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Occurrence, Fate and Distribution Behaviors of Organic Contaminants, Perfluorinated Alkyl Acids and Phthalic Acid Esters, in Wastewater Effluent and the Housatonic River Estuary.

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Occurrence, Fate and Distribution Behaviors of Organic Contaminants,
Perfluorinated Alkyl Acids and Phthalic Acid Esters, in Wastewater Effluent
and the Housatonic River Estuary.

Joanne Ailsa Elmoznino, PhD

University of Connecticut, 2016

ABSTRACT

The Long Island Sound (LIS) is a local asset strained by population growth and development. While regulatory effort has focused on nutrient pollution, no regulations govern organic micropollutants though they may be toxic at low concentrations. Target micropollutants in this study are two compound classes with several homologues: Perfluoroalkyl acids (PFAAs), used for stain repellency and non-stick cookware, are extremely persistent, and phthalic acid esters (PAEs) are plasticizers of high production volumes. Both are ubiquitous environmental pollutants which have come under intense scrutiny due to both bioaccumulative and endocrine disruptive properties.

The overarching aims of this doctoral thesis were to determine the occurrence, distribution and fate of PFAAs and PAEs from wastewater (WW) effluent impacting the CT shoreline region of the LIS. Discharges of domestic and industrial origin were identified, for the first time, as major sources of PFAAs and PAEs to this region. The annual mass flow of target PFAAs to LIS watershed from WW point-sources was estimated to be in the range of 50 - 530 kg/year; the mass flow of PAEs was estimated at between 60 – 270 kg/year.

Joanne Ailsa Elmoznino - University of Connecticut, 2016.

Analysis of sediments, suspended particulate matter (SPM) and water along the Housatonic River found that while PFAA partitioning to solid phases increased with perfluoroalkyl chain length, little was lost to sediments. PFAA mass flow was conserved into the estuary displaying a dilution gradient consistent with source waters mixing with estuary waters. Conversely, PAEs were detected in higher concentrations in the estuary surface waters than in the river, indicating the estuary to be a trap for these contaminants.

Total PFAA concentrations decreased with increased river flow suggesting the importance of point sources. Average total PFAA mass flux to the LIS from the Housatonic was estimated at 60 – 90 g/day in low flow hydrology, and 200 - 340 g/day during the high flow regime. A high concentration pollution event indicated the continued industrial use of restricted PFAAs in this region.

SPM was found to play an important role in contaminant transport in effluent and receiving waters. Partitioning parameters derived for SPM and sediments in the field were 1-2 orders of magnitude greater than experimentally derived literature values, but consistent with other field observations. The higher order partitioning to SPM than sediments highlights the role of SPM as a vector of contaminants to the food chain.

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and the Housatonic River Estuary.

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APPROVAL PAGE

Doctor of Philosophy Dissertation

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Chapter 1

Introduction to Dissertation Research

1.1 Background and research motivations

Over the past few decades, the persistent, bioaccumulative and toxic (PBT) natures, of many organic chemicals used copiously worldwide in numerous products and applications, have been realized. Twelve of the most harmful persistent organic pollutants (POPs) were recognized as such, with their use being either banned or limited by the Stockholm Convention on Persistent Organic Pollutants, an international treaty signed in 2001 (1). More recently, the overwhelming evidence of numerous, human activity derived chemical contaminants prevalent in the environment, has led much of the scientific community to prioritize research in the area of 'Emerging Organic Contaminants'. The term Emerging Organic Contaminant (EOC) describes pollutants, also referred to as micro-organic contaminants, that although are being increasingly detected in the environment, the ramifications of their presence are not fully understood because the transport, fate and distributions, and full range of toxic effects, are still largely unknown. Consequently the regulatory framework controlling their usage and allowable inputs into coastal waters are not easily established. EOCs are comprised of a wide array of chemical compounds, used by society in a wide range of industrial and domestic purposes, for example, pharmaceuticals and veterinary medicines, personal care products, surfactants, plasticizers, preservatives, food additives and pesticides,

with some of the most prevalent EOCs detected in ground water being those termed as “life-style’ compounds; antibiotics, anti-inflammatories, and caffeine (2).

The target compounds in this study are known EOCs. Part I of this dissertation is concerned with perfluoroalkyl acids (PFAAs), surfactant molecules that have gained widespread uses mainly as repellants. As recent as the early 2000s, researchers began to detect the global presence of these new PBT compounds, specifically long chain perfluorinated acids and their conjugate bases, including perfluorinated carboxylic acid/carboxylates (collectively known as PFCAs) and perfluorinated sulfonic acid/sulfonates (PFASs) (3). These compounds are extremely stable in both industrial applications and, once emitted either directly from industrial sources or through the discharge of domestic wastewater from the breakdown of consumer products, they are also very stable in the environment, resistant to all biotic and abiotic degradation processes. PFAAs have been shown to bioaccumulate and biomagnify in the environment, particularly in aquatic organisms such as fish and fish-eating predators, and have been shown to elicit toxic effects in animal studies. They are also ubiquitously detected in the blood of wildlife and humans worldwide (3). There is much that is still not known regarding the origins, distribution and biogeochemical behavior of PFAAs in the aqueous environment, which is due in part to a lack of data regarding contamination and distributions at environmentally relevant levels.

While the major portion of this dissertation research is concerned with the occurrence and fate of PFAAs in the Connecticut (CT) shoreline region, a concurrent study was also performed to investigate the occurrence and potential impacts of Phthalic Acid Esters (PAEs), plasticizers that are used mainly as additives in the

manufacture of soft polyvinyl chloride (PVC) plastics. They have received widespread attention in recent years due to compounds in this class identified as endocrine disrupting chemicals (EDCs); having the potential to elicit hormonal responses which could severely affect the reproductive growth and development of a fetus if exposed at a sensitive time in gestational development. The potential impact of PAEs on human health has been reported widely in media, drawing the attention and concerns of the general public due to the presence of these compounds in numerous consumer products that infants may have oral contact with such as toys and baby bottles. During the past 40 years, the high production volume of PAEs (mainly in the production of PVC and the numerous commercial uses of) has led to their ubiquitous presence as environmental pollutants, even in remote marine environments (4). While there are some provisional guidelines concerning the recommended levels of several PFAAs in drinking water, there are currently no regulations concerning acceptable discharge levels of, or any regular monitoring in the aquatic environment of, either PFAAs or PAEs.

The LIS is an economically important urban sea, utilized recreationally, industrially and commercially, with a population of approximately 16 million people living and working on its shores, thus understanding any potential impacts from anthropogenic activities is of critical importance to many in this region. In addition, the understanding of organic contaminant biogeochemistry in aquatic systems is still limited by the lack of field data describing distributional behavior in different systems. The partitioning of organic pollutants describes the tendency of a compound to be found in, or to move to or from, an environmental phase or compartment. Understanding distribution behavior

and influencing factors, and obtaining reliable expressions for partitioning processes, is key for environmental modeling; to be able to predict the potential occurrence in and impact to local ecosystems, to predict the potential for human exposure, and to perform risk assessment analysis (5).

1.2 Hypotheses and objectives

1.2.1. Hypotheses

Wastewater effluent discharge from wastewater treatment plants (WWTPs) has been identified as a major source of PFAAs to the aquatic environment (6) (7) with concentrations reported in the ng/L to µg/L range (see Chapter 2) however no data has been published in the peer-reviewed literature on the presence of PFAAs in Connecticut wastewater facilities, with discharges that directly impact the LIS watershed. Therefore, detecting PFAAs in wastewater effluent in CT shoreline WWTPs is the first overarching goal of this dissertation.

H1: There are detectable concentrations of PFAAs in WWTP final effluents, discharging into the Long Island Sound CT shoreline region.

In receiving waters, the PFAAs will tend towards different environmental compartments, including water, suspended or sedimentary particulate matter or biota, as a function of their physical chemical properties. Longer chained PFAAs (>6 fluorinated carbons) have been shown to partition to solid phases such as sediments,

as a function of chain length. Higgins and Luthy, in their seminal 2006 paper, described the partitioning of perfluorocarboxylates and perfluorosulfonates with 7 or more fluorinated carbons, to five different freshwater riverine and lacustrine sediments, as a process influenced by sediment and solution specific parameters (8). The organic carbon content of the sediment was found to be a dominant parameter affecting sorption, with sorption increasing in relation to the increasing organic carbon content (or fraction of organic carbon (f_{oc})) of the sediments. PFAA sorption was also reported to increase with increasing solution calcium and hydrogen ion concentrations, and to be a function of chain length, with each CF_2 moiety contributing 0.5 – 0.6 log units to the measured distribution coefficients.

Partition coefficients reported by Higgins and Luthy were derived using laboratory based sorption isotherm experiments. In contrast, several studies have reported field based partitioning constants, describing water-sediment partitioning (K_d), or water-sediment organic carbon partitioning (K_{oc}) to be greater than those derived by Higgins and Luthy, by an order of magnitude (9, 10). Furthermore, the extent of PFAA partitioning to suspended particulate matter (SPM) was reported to be of greater extent than that describing sorption to bed sediments in the same field study sites of Tokyo Bay and the River Seine, Paris (9, 10). As a consequence, the distribution coefficients that should be used for assessing environmental fate do not have a clear consensus. In addition, partitioning parameters may vary with location; therefore deriving field based partitioning parameters for the CT shoreline region is the second overarching goal of this dissertation. The role of SPM is of particular interest in this dissertation research, as SPM provides a crucial link between water, bed sediments and the food chain, so can

control both the transport and the biological impacts of associated contaminants, and in doing so, can play an important role in their biogeochemical cycling (11).

While the wastewater treatment process removes over 99% of suspended solids, the wastewater effluent stream itself contains a small fraction of effluent derived SPM (efSPM). As efSPM is transported into receiving waters, it constitutes a parameter of organic pollution and may itself perform an important role in facilitating the transport of more hydrophobic pollutants through the WWTP and into receiving waters.

H2: In effluent waters, partitioning between dissolved and particulate phases will be a function of the physicochemical properties of each chemical compound, with the shorter chain or more soluble PFAAs found predominantly in the dissolved phase of the final effluent waters. WWTP effluent suspended particulate matter (efSPM) is a vector for longer fluoro-alkyl chain PFAAs into receiving waters, with the role of efSPM increasing with perfluoro-alkyl chain length.

Hypotheses 1 and 2 will be discussed in Chapter 3: Occurrence and Partitioning Behavior of Perfluoroalkyl Acids in Wastewater Effluent Discharging into the Long Island Sound and Tributaries.

WWTPs are known point sources of fluorochemicals to receiving waters. The fate of PFAAs released from WWTPs into a receiving riverine system was investigated in the

Glatt Valley watershed in Switzerland by Huset *et al.* in 2008 (12). The mass flows of PFCAs (with between 6 - 10 fluorinated carbons) and PFSAAs (with between 4 – 10 fluorinated carbons) from seven WWTPs were measured and compared to the measured mass flows within the 35 km fresh waters of the Glatt River. The authors found perfluorochemical mass flow to be conserved, with input from the WWTPs to be additive, and removal within the Glatt River to not be significant, for PFCAs with between 6 – 8, and for PFSAs with 4, 6 and 8 fluorinated carbons. PFAAs with longer perfluoroalkyl chains were only detected in effluent samples at low concentrations. Given the solubility of PFAAs with less than 8 perfluoroalkyl moieties, this behavior would be predicted.

Investigating the fate of PFAAs entering receiving waters from CT WWTPs is the third overarching goal of this dissertation. Unlike the Glatt River, the mass flow of PFAAs into LIS shoreline harbors and major tributaries will be from fresh (WWTP or riverine) waters, to saline estuarine waters. From the data from Higgins and Luthy, it appears that the perfluoroalkyl chains and hydrophobic interactions facilitate sorption of PFAAs. For nonpolar and weakly polar organic contaminants, increasing salinity results in increased sorption to estuarine particulates due to the “salting out” effects of electro-restriction, whereby the increased presence of dissolved inorganic salts interacting with water molecules effectively reducing ‘free’ water available in which the contaminants can dissolve, resulting in an apparent decrease in the aqueous solubility of the contaminant (13). As the “salting-out” effect increases exponentially with increasing salt concentrations (13), the mass flow of organic contaminants along a salinity gradient would not be predicted to be conservative.

Greater contaminant sorption to particulate organic matter is also attributable to the reduction in the overall negative surface charge of natural organic matter by the interactions with the common seawater cations (i.e., Na^+ , K^+ , Mg^{2+} , Ca^{2+}) thus for anionic species, lowering electrostatic repulsion effects that may be inhibitory to sorption (14). Increased sorption of longer chain PFAAs to sediments with increasing calcium ion (Ca^{2+}) concentration was reported by Higgins and Luthy (8). Sorption of perfluorooctane sulfonic acid to sediments was also reported to increase with Ca^{2+} concentrations by You *et al.* (15) and with Mg^{2+} and Ca^{2+} by Chen *et al.* (16). The role of divalent cations in PFAA sorption has been postulated to be that of facilitating sorption by creating a bridge between the negatively charged organic matter surface and the anionic PFAA functional group (8, 15). This proposal is supported by the additional observation reported by Higgins and Luthy that PFAA distribution coefficients did not significantly increase with increasing solution concentrations of the monovalent sodium ions (Na^+) (8). Similar sorption behaviors are reported for other anionic surfactants, such as the linear alkylbenzenesulfonates (LAS), which were reported to partition to sediments as a function of sediment organic carbon content (f_{oc}), and Ca^{2+} (17). Therefore, in this dissertation research, it was predicted that the PFAA mass flow along the salinity gradient would not be conservative, as previously reported for the freshwater Glatt River Valley by Huset *et al.*

The fate of PFAAs entering receiving waters in this region was investigated with a focused study on the Housatonic River, the second largest tributary river to the LIS, with six WWTPs discharging directly into the river at locations along the salinity gradient of the lower river, which was selected as the area for this field study

H3: WWTP discharge is a major source of PFCs to the Housatonic River, where mass transport will be non-conservative due to increasing sorption to solid phases (sediments, SPM) along the salinity gradient.

The biogeochemical dynamics of PFAAs were investigated in the heavily urbanized River Seine, (Paris, France) by Labadie and Chevreuil, where WWTP point sources were also proposed to be the predominant sources of PFAAs to the river due to the negative correlation of total PFAA levels with river flow rate (18). The mass flow of PFAAs along the Housatonic are predicted to behave similarly, with lower PFAA concentrations in riverine waters during the spring high river flow regime in comparison to the summer low flow hydrology. Individual PFAAs that remain in solution at higher salinities are expected to behave similarly under low and high flow hydrology's, and mass flow is predicted to be conserved as observed in the Glatt River, however it is predicted that with a larger Housatonic River plume under spring high flow, the PFAA exposure areas in the LIS of individual PFAAs influenced by 'salting-out' will increase relative to the summer low flow regime.

H4: Concentrations of PFAAs in the Housatonic River waters will be negatively correlated with river flow however increased discharge during the spring high river flow hydrology will result in increased exposure to the LIS of individual PFAAs influenced by 'salting-out' during summer low flow.

The fate of PFAAs entering the Housatonic River Estuary will be further investigated in this study, by measuring the distribution of individual PFAA congeners between the three phases (water, sediment and SPM) along the salinity gradient. Parameters obtained for describing partitioning are predicted to; 1. Be greater for SPM than for sediments obtained from the river bed, 2. To increase with increasing salinity and 3. To increase with increasing perfluoroalkyl chain length. In addition, oysters were selected to be used in a pilot study to investigate PFAA uptake due to their abundance in bed sediments situated in the Housatonic River mouth. As filter feeding bivalves, which ingest suspended particulates, oysters can be used to assess the importance or impact of the SPM bound contaminants (19).

H5: In receiving waters PFAAs will partition between dissolved, SPM, sediment phases, and biota as a function of perfluoroalkyl chain length.

H3, H4 and H5 will be addressed in Chapter 4: Occurrence and distribution of PFAAs along the Housatonic River estuary under contrasting hydrological regimes.

Finally, an additional concurrent study was performed to assess the occurrence and distribution behaviors of phthalic acid esters (PAEs) in wastewater effluent and along the Housatonic River Estuary. Wastewater effluent discharge has also been identified as a major source of PAEs to the aquatic environment (20) however no data

has been published in the peer-reviewed literature on the presence of PAEs in wastewater discharges that impact the LIS watershed from CT shoreline WWTPs. PAEs are neutral organic compounds; the target PAEs range in molecular mass and physical chemistry, as the PFAAs, from smaller more soluble PAEs to larger more hydrophobic congeners. For hydrophobic organic contaminants, solution chemistry has also been shown to be an important parameter in their distribution behaviors. Of particular interest in this dissertation research is the influence of dissolved organic carbon (DOC), which has been reported to correlate significantly with organic pollutant partitioning, suggesting it plays an important role in transport and in decreasing the efficiency of WWTPs in removing hydrophobic contaminants (21).

H6: There are detectable and environmentally significant concentrations of PAEs in WWTP final effluents and in the receiving waters of the Housatonic River. PAE partitioning between effluent, riverine and estuarine suspended particulate matter (SPM) and the dissolved phase is a function of, SPM-organic matter content, dissolved organic carbon (DOC), and salinity (for riverine/estuarine SPM) in conjunction with target compound physicochemical properties. Partition coefficients are expected to:

- **Increase in direct proportion to decreasing solubility/increasing K_{ow}**
- **Increase with increasing salinity**
- **Decrease with increasing DOC**

H6 will be addressed in Chapter 5: Occurrence of PAEs in the Final Effluent from Several CT WWTPs and in the Housatonic River Receiving Waters.

1.2.2. Objectives

The main objective of this dissertation is to improve understanding of the occurrence, distributions and fate of PFAAs and PAEs in a sewage impacted coastal marine environment. This includes the derivation of field based physical parameters, which are of critical importance for better modeling of pollutant transport and for the assessment of risk to ecosystems and human health. The initial goal of this research was to determine the presence of target PFAAs and PAEs in CT wastewater effluent that is discharged into the CT shoreline region of the Long Island Sound or into one of its major tributaries, thereby establishing baseline concentrations, as these organic micro-pollutants have not been previously measured in this location.

- **Obj. 1:** Undertake a survey of the occurrence of PFAAs/PAEs in samples of final effluent from 12 of the Connecticut WWTPs which discharge into the LIS or one of its major tributaries, and determine partitioning constants for target compounds in the dissolved and the SPM phases in final effluent.

Once the presence of target compounds was established, the overarching goal of this research was to determine how the compounds behave in effluent, riverine and estuarine waters, with specific attention to the distributions between water, sediments and suspended particulate matter (SPM) as a function of salinity, dissolved organic carbon concentrations, and particulate organic carbon content and chemical

characteristics, then comparing these behaviors across the chemical compound class to quantify the extent to which physicochemical properties affect fate and distribution behavior.

- **Obj. 2:** Study of occurrence and distributions of target compounds along a salinity gradient by conducting a field survey along the Housatonic River (the second largest river bringing fresh water to the LIS) with specific attention given to determining occurrence and partitioning between water, SPM, sediment phases, and to the detection in and partitioning to biota, specifically oysters, to be deployed at several stations in the mouth of the river estuary. Field observations are to be compared with laboratory based experiments, investigating water-SPM partitioning of PFAAs.

The objectives of this dissertation are addressed in the following chapters;

- Chapter 1 outlines the hypotheses and objectives of this dissertation.
- Chapter 2 gives a review of the current literature concerning PFAAs and addresses current understanding on the role of suspended particulate matter of the fate and transport of organic pollutants.
- Chapter 3 presents data on the occurrence and partitioning behaviors of PFAAs in the final effluent from several CT WWTPs.
- Chapter 4 presents data on the distributions of PFAAs in the receiving waters of the Housatonic River and estuary under contrasting hydrological regimes, providing insight into the transport and biogeochemistry of PFAAs along a salinity gradient.

- Chapter 5 presents data on the occurrence and distributions of PAEs in WWTP final effluent, and the impact on the receiving waters of the Housatonic River and estuary.
- Chapter 6 is an overall summary of the important findings from this study, followed by recommendations for future research.

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Chapter 2

Perfluoroalkyl Acids: An overview of the current literature

Abstract

Perfluoroalkyl acids (PFAAs) have been used in numerous consumer products and industrial applications since the 1950's. Their unique physicochemical properties include water and oil repellency, stability and resistance to attack by most acids and bases. Yet, it is these same properties that have also imparted the specific characteristics of a pollutant of concern, namely, that they are persistent in the environment, bioaccumulative, and toxic. By early 2000s it became apparent that PFAAs could be detected in almost every environmental compartment worldwide, including the blood of wildlife and humans. How the globe became contaminated has been the subject of intense scientific inquiry, and two major transport pathways have been proposed; oceanic transport of soluble PFAAs emitted directly into the aquatic environment, and the atmospheric distribution of volatile precursor compounds. Many questions remain regarding the origins, distribution and biogeochemical behavior of PFAAs in the aqueous environment, and there is a lack of data regarding contamination and distributions at environmentally relevant levels. The objective of this chapter is to provide an overview of the current literature regarding the concerns of PFAAs, and their occurrence and fate in the aquatic environment.

2.1 Introduction

Perfluoroalkyl compounds (PFCs) are an example of commercially useful anthropogenic compounds which have gained widespread use due to their distinctive chemical characteristics; these include water and oil repellency, chemical and thermal stability and inertness, and surface active properties in both aqueous and solvent systems that are unparalleled (1). PFCs are characterized by a partially or fully fluorinated alkyl chain and a terminal functional group, such as carboxylate, sulfonate, sulfonamide, phosphate or alcohol. PFCs have been used commercially since the 1950's in a wide range of products and applications including their use as processing additives in the manufacture of fluoropolymers such as Teflon®, as surfactants for surface coatings of consumer products including textiles, furniture and paper products, including paper products approved for food contact, and in products such as Scotch guard®, as emulsifiers in the formulations of paints, personal care products and pesticide formulations, electronics and electroplating; photo imaging; hydraulic fluids; and for use in fighting hydrocarbon fueled fires as aqueous film forming firefighting foams (2, 3, 4, 5, 6, 7).

The most commonly studied PFCs are the anionic perfluoroalkyl acids (PFAAs); particularly the perfluorocarboxylic acids (PFCAs) and the perfluorosulfonic acids (PFSAs) (Figure 2.1). Of these two chemical groups, the 8-carbon chained compounds, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are widely recognized as two of the newest persistent organic pollutants (POPs) of greatest concern due to their global presence and distribution, and are the PFC species generally detected in the highest concentrations in all environmental samples (6, 8).

Neutral PFCs include compounds such as perfluoroalkyl sulfonamide (FASAs), perfluoroalkyl sulfonamidoethanols (FASEs) and the fluorotelomer alcohols (FTOHs). These compounds are characterized by lower water solubilities and greater volatility than the anionic PFCAs and PFSA, and have been shown to degrade in the environment to the final, environmentally persistent, degradation products, PFCAs and PFSA (9, 10, 11).

The Organization for the Economic Co-Operation and Development (OECD) listed fluorinated organic compounds C₅ to C₁₈, which includes PFOS and PFOA, as high production volume (HPV) chemicals, manufactured in the USA with a volume exceeding 1 million pounds per year prior to 2000 (2). Mounting evidence of the persistent, bioaccumulative and toxic (PBT) nature of PFCs lead to the voluntary discontinuation of PFOS and related compounds by the principal manufacturer, 3M, working with the US Environmental Protection Agency (EPA), between 2000 and 2002, as well as inclusion of these compounds to Annex B of the Stockholm Convention on POPs in 2009, thereby restricting manufacture and use. The European Union (EU) prohibited the general use of PFOS and its derivatives in June 2008 (12). In the 2010-2015 PFOA stewardship program, the US EPA worked with the leading PFC chemical based industries to reduce emissions of PFOA and long-chain PFCAs by 95% in 2010, working towards elimination of long-chain PFCAs by 2015 (13). However PFCs emissions continue to be of global concern as production in developing regions has increased in order to fulfill industrial demands (6) with an estimated PFOS production of 1000 t per year since 2002 (14). In addition, neutral precursor compounds continue to be produced (7).

The aim of this chapter is to provide an overview of the current literature regarding the concerns of PFAAs, and their occurrence and fate in the aquatic environment, in order to illustrate the gaps in the current scientific knowledge which this dissertation aims to address. Thus the objectives of this chapter are to:

- Give an introduction to PFAAs, discussing the history of production, chemistry and environmental behavior, and the PBT nature of PFAAs.
- Outline the reported mechanisms of long-range transport and summarize data on reported environmental concentrations, including PFAAs in wastewater point-sources, and in riverine and estuarine receiving waters.
- Summarizing data regarding biogeochemistry of PFAAs in the aquatic environment, focusing on current literature regarding the role of suspended particulate matter.

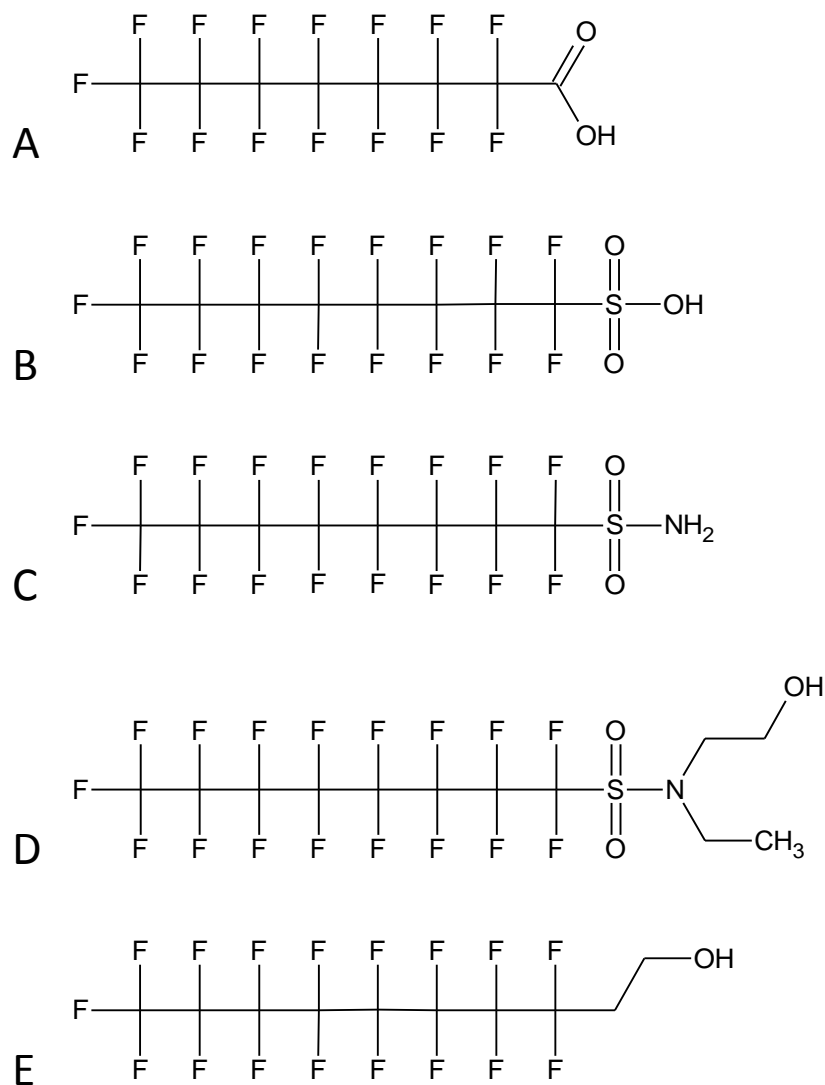


Figure 2.1: Generic chemical structures of perfluorinated compounds. A- Perfluorocarboxylic acids (ex. Perfluorooctanoic acid (PFOA)). B- Perfluorosulfonic acids (ex. Perfluorooctane sulfonic acid (PFOS)). Neutral PFCs include C- Perfluorosulfonamide (FASAs), D- Perfluorosulfonamidoethanol (FASEs) and E- Fluorotelomer alcohols (ex. 8:2 FTOH). The $n = 8$ linear carbon structure is shown, however $n = 4 - 14$ PFCAs and $n = 4 - 10$ PFSAAs are compounds included in this study.

Table 2.1: Target PFAAs- Chemical names and molecular formulas

PFAAs		
Acronym	Full name	Chemical Formula
<i>PFCAs</i>		
PFBA	Perfluorobutanoic acid	$C_4F_7O_2$
PFPA	Perfluoropentanoic acid	$C_5F_9O_2$
PFHxA	Perfluorohexanoic acid	$C_6F_{11}O_2$
PFHpA	Perfluoroheptanoic acid	$C_7F_{13}O_2$
PFOA	Perfluorooctanoic acid	$C_8F_{15}O_2$
PFNA	Perfluorononanoic acid	$C_9F_{17}O_2$
PFDA	Perfluorodecanoic acid	$C_{10}F_{19}O_2$
PFUnA	Perfluoroundecanoic acid	$C_{11}F_{21}O_2$
PFDoA	Perfluorododecanoic acid	$C_{12}F_{23}O_2$
PFTTrA	Perfluorotridecanoic acid	$C_{13}F_{25}O_2$
PFTeA	Perfluorotetradecanoic acid	$C_{14}F_{27}O_2$
<i>PFSAs</i>		
PFBS	Perfluorobutane sulfonic acid	$C_4F_9SO_3$
PFHxS	Perfluorohexane sulfonic acid	$C_6F_{13}SO_3H$
PFHpS	Perfluoroheptane sulfonic acid	$C_7F_{15}SO_3H$
PFOS	Perfluorooctane sulfonic acid	$C_8F_{17}SO_3H$
PFDS	Perfluorodecane sulfonic acid	$C_{10}F_{21}SO_3H$

2.2 History of Production

PFC production began in the late 1940s by either telomerization or electrochemical fluorination (ECF) techniques (15). Telomerization creates exclusively linear isomers of even numbered chained PFCAs as well as fluorotelomer alcohols (FTOHs); volatile PFC species that are precursors of PFCAs. (3). In the ECF process a straight chain hydrocarbon is reacted with hydrofluoric acid and an electric current

passed through so all hydrogen molecules are replaced by fluorine, generating perfluorocarbons of even and odd perfluoroalkyl chain lengths as well as branched chain isomers. Despite lower yields and formation of side products, ECF was used predominantly for commercial production of perfluorooctane sulfonyl fluoride (POSF) based products, such as PFOA and PFOS, in the USA due to the relatively low costs (2, 16).

The 3M Company was the major producer of POSF. While production volumes are reported to be difficult to estimate due to the proprietary nature of the information and production responses to recent regulations, the total global, cumulative production was estimated to be 96,000 t in the peak years (1970-2002) (6, 14). Current production volumes have been estimated at 1000 t per year, as following the 2002 discontinuation by 3M, other companies began production to fulfill market demands (14). Global emission estimates of PFCAs were recently quantified by Wang *et al.* (17), based on the life-cycle of PFCA and POSF based products, reporting emissions of 2610 - 21400 t between the years 1951 - 2015, and a predicted emission volume of 20 - 6420 t in the years 2016 - 2030, along with a geographical shift of industrial sources from North America and Europe, to Asian countries such as China. In Asia, the annual production of PFOS and POSF has increased from <50 t prior to 2004, to >200 t from 2005 on (18). In addition, there are also concerns regarding the continued production and use of numerous other PFCs, such as the more volatile precursor compounds including as fluorotelomer alcohols (FTOHs) and perfluoroalkyl sulfonamides (FASAs), which are capable of long-range atmospheric transport (10). Sources of PFAA are also found to vary for the different homologues; approximately 100% of PFOA emissions (1951-2002)

were reported as attributable to direct-release during the life-cycle of products in which PFOA was used as an ingredient or is present in the product as an impurity, whereas in contrast 9-78% of environmental contaminations of PFDA were attributed to the historical emission of, and subsequent degradation of, precursor compounds (17).

2.3 Physical Chemistry and Environmental Fate

The small size and high electronegativity of the fluorine atom produces a highly polar carbon-fluorine bond with a large bond energy; the C-F bond is one of the strongest bonds in organic chemistry. The shielding provided by three non-bonding pairs of electrons around each fluorine atom, confers remarkable stability to PFAAs, including resistance to degradation by acids, bases, oxidants, reductants, as well as environmentally relevant degradation processes (3, 19, 20). The presence of the charged moiety, such as the carboxylic or sulfonic acid group, increases solubility resulting in surfactant properties. PFAAs are thus hydrophobic and oleophobic in nature, repelling both water and oil, as well as being thermally stable and one of the most effective surface tension reducing surfactants commercially available. (1).

The PFCAs and PFSAAs which are most commonly studied range in perfluoro-alkyl chain length from 4-14 carbons for the PFCAs, and from 4 to 10 carbons for the PFSAAs. Detailed information regarding the chemical names, acronyms and chemical structures is given in Table 2.1. The physical-chemical properties of PFCs was recently reviewed by Rayne and Forest (21) and Ding and Peijnenburg (8). For most PFAAs, basic physicochemical data is scarce, or is the subject of controversy. PFSAAs are believed to be strong acids, therefore at all relevant aqueous environmental conditions, PFSAAs will

be in the ionized form (8). The pK_a values of PFCAs, considered to be weaker acids, is however a subject of much debate, with reported values for PFOA ranging from ~ 0 to 3.8 (21, 22). More recent analyses do however find pK_a 's values of between 0 and 1 for PFCAs and lower for the PFSA's (23).

Experimental values of the aqueous solubilities of PFAAs are also scarce (8). Data from the 3M Environmental Laboratory found the solubility of PFBS to be 52.6 – 56.6 g/L (8). Solubilities of PFOS have been reported 550 mg/L and 498 mg/L in pure water at 24 and 20 °C respectively, and 21.8 mg/L in seawater at 20 °C (8 and references therein). The solubility of PFOA is also a subject of debate, regarding whether values are true solubilities or rather are due to micelle microdispersion (8) however solubility is unlikely to be a concern at typical environmental concentrations ($< \text{mg/L}$) (21). PFAAs have low vapor pressures which are also reported to decrease with increasing chain length, therefore volatilization from surface waters or soils is not expected to be a significant environmental fate (21).

The octanol/water partition coefficient (K_{ow}) describes the ratio of the concentration of a chemical found in the n-octanol phase compared to the concentration found in the underlying water phase at equilibrium and at a specified temperature. Using n-octanol as a surrogate for fat (lipids) or organic matter relates K_{ow} to estimates of bioaccumulation factors and soil or sediment adsorption coefficients, therefore experimentally derived K_{ow} values are important parameters in assessing the environmental behavior and fate of organic pollutants. Experimentally derived PFAA K_{ow} values are however scarce due to the hydrophobic and lipophobic nature of the molecules, which results in the formation of several immiscible layers when added to

octanol/water systems (5, 8). As PFAAs are proteinophilic, thus partition preferentially into protein rather than lipids as do classical POPs, it is considered in this case that K_{ow} values do not allow for the estimation of environmental partitioning of these compounds (9). Estimates of K_{ow} values for several PFAAs were reported by Jing *et al.* (24) using ion-transfer cyclic voltammetry; data from this study is shown in Table 2.2. Jing *et al.* concluded that the perfluoroalkyl carboxylates are ~2 orders of magnitude more lipophilic than the hydrocarbon counterparts, which the authors attribute to reduced hydrophilicity of the anion group due to electron withdrawing effect of the adjacent perfluoroalkyl group.

The distribution between soil or sediment and water is described by the partition coefficient (K_d), describing the ratio of concentrations of PFAA in both solid and water phases at equilibrium. The organic carbon/water distribution coefficient (K_{oc}) is determined by dividing the K_d by the total organic carbon content. Given the hydrophobic and oleophobic nature of the perfluoroalkyl chain, with increasing K_{ow} values with increasing chain length (Table 2.2), it is likely that hydrophobic effects will influence PFAA partitioning behavior between water and organic matter; effects that are also expected to increase with increasing chain length. Due to the anionic nature of the sulfonate or carboxylate head groups, electrostatic effects will also influence PFAA sorption. Several studies have reported on the sorption behavior of PFAAs in soils and sediments (25, 26, 27, 28, 29). Partitioning to solids becomes measurable for longer-chain PFAAs ($>C_7$), and is influenced by both sediment characteristics as well as solution chemistry, however the governing feature influencing sorption was determined to be the perfluoroalkyl chain length, with each $-CF_2-$ moiety increasing distribution

coefficients by 0.50-0.60 log units as reported by Higgins and Luthy, 2006 (25). This result is consistent with the 0.59 log unit difference in log K_{ow} values reported by Jing *et al.* (24) (Table 2.2).

In summary, at typical environmental concentrations, PFAAs are projected to dissociate in the aquatic environment, have limited volatilization capabilities due to their low vapor pressures, and limited sorption to particulates, thus the major quota of PFAAs emitted into the aquatic environment is expected to exist in in surface receiving waters and oceans (8). Longer-chain ($>C_7$) PFAAs, with log K_{oc} values >2 , do however show low to increasing tendency to partition to solids, with increasing CF_2 chain length, therefore sediments as well as deep ocean are potential environmental sinks of PFAAs (7)

Table 2.2: Experimentally derived log K_{ow} values for several PFAAs from the study by Jing *et al.* (2009) (24)

PFAA	Log K_{ow}
PFBA	-0.52
PFPA	0.09
PFHxA	0.70
PFHpA	1.31
PFOA	1.92
PFNA	2.57
PFDA	2.90
PFOS	2.45

2.4 Global Distribution Transport Pathways

By early 2000s it became apparent that the unique properties of PFCs which have made them largely irreplaceable in a wide range of industrial applications and consumer use products, had also led to their global detection in every environmental compartment (air, water, soils etc.), and ubiquitous detection in the blood of wildlife and humans worldwide, including those in remote locations, such as the Arctic (6, 20, 30, 31). Measurable levels of PFOS in the tissues of fish, birds, and marine mammals in both industrial regions such as the North American great lakes region and the Mediterranean Sea, and in remote Arctic regions were first reported by Geisy and Kannan in 2001 (20). PFCs appear to undergo “global distillation” in the same way classical POPs have been shown to be transported from the anthropogenic influences of the temperate regions to the polar regions where they, much like PCBs and DDT, have been found to accumulate in animals such as polar bears and seals (32) (33).

How the globe became contaminated with PFAAs has been the subject of intense scientific scrutiny since the 2001 study of Geisy and Kannan (20), and two major transport pathways have been proposed. The first pathway involves the oceanic transport of water soluble PFAAs emitted directly into the aquatic environment from point and non-point sources. Specific point sources of PFAAs include industrial emissions from fluorochemical manufacturing processes (34), or from industries that specifically utilize the properties of fluorochemicals in their products, such as repellants for carpet and textile manufacturing (35). Effluent from wastewater treatment plants (WWTPs) is also a known point source of PFAAs to aquatic ecosystems, discharging residential derived fluorochemicals released from residuals in consumer products (36,

37). Non-point sources of PFAAs include run-off from urban areas (38), and from specific industries where fluorochemicals are utilized in products such as firefighting foams such as military and commercial airports (39). As PFCAs and PFSAAs are extremely persistent under ambient environmental conditions, they are amenable to long distant oceanic transport. Recent research surveys from Northern Europe, Atlantic and Southern oceans (40) and in the North Pacific to the Arctic Ocean (41) reported on the presence and spatial distributions of PFAAs, indicating that industrial regions were sources of PFCs, such as the North American and East Asian coasts.

The second pathway involves the atmospheric transport of volatile precursor compounds from point source emissions, and subsequent degradation to PFCAs and PFSAAs followed by removal from air to land via wet (rained-out) or dry deposition. A number of volatile PFC compounds have been shown to degrade abiotically to PFAA final degradation end products, including fluorotelomer alcohols (9), perfluorinated sulfonamide alcohols (FOSEs) (10), as well as recent research indicating that several PFCs may form PFCAs via atmospheric oxidation, including fluorotelomer olefins (42), fluorotelomer iodides (43) and fluorotelomer acrylates (44).

Ellis *et al.* (9) proposed that FTOHs degraded in the atmosphere via reaction with hydroxyl radicals as the main source of PFAAs in the Arctic. This proposal was modeled by Wallington, *et al.* (45), taking global 8:2 FTOH emission estimates to determine the expected concentrations of PFOA in the Arctic using the IMPACT model. Results from this study predicted that 8:2 FTOH, with a measured half-life of 20 days, would be globally distributed and the molar yields of PFOA would be in the correct order of magnitude to explain the levels of PFCAs measured in Arctic fauna. Atmospheric

concentrations of FTOHs were measured by Shoeib *et al.* (46) during a research cruise through the North Atlantic and Canadian Archipelago. Results from this survey confirmed the Wallington *et al.* (45) model predictions of efficient, long-range atmospheric transport and widespread distribution of FTOHs in the Arctic region.

2.5 Bioaccumulation in Aquatic Organisms

The bioaccumulation potential of organic contaminants refers to the relative concentrations of the chemical in an aquatic organism relative to the environment in which they inhabit. This can be defined using the bioconcentration factor (BCF, bioaccumulation factor (BAF), or the biomagnification factor (BMF). The BCF describes the ratio of the concentration of a chemical in a test organism compared to that of the surrounding water, considering only the water-borne exposure routes of dermal and respiratory uptake. The BAF describes the ratio of the concentration of a chemical in an organism to that in the water, accounting for all possible routes of exposure including dermal, respiratory and dietary uptake. BAF values are usually field derived coefficients. BMF describes the ratio of the concentration of a chemical in an organism relative to that in its diet, and is usually used to describe predator-prey relationships.

Unlike most POPs which tend to accumulate in lipids, PFAAs are proteinophilic, thus tend to accumulate in blood, liver and kidneys (47, 48, 49). This is a major contributing factor to the significantly smaller data set regarding the levels of PFAAs in wildlife compared to other emerging halogenated POPs such as polybrominated diphenyl ethers (PBDEs), since existing samples from other more researched halogenated compounds, such as polychlorinated biphenyls (PCBs), can be utilized for

PBDE analysis; PFAAs however required different extractions and often other tissue compartments (37). As the exposure routes and accumulation mechanisms are also different to classical hydrophobic POPs, the use of traditional K_{ow} –based models to predict bioaccumulation is neither appropriate nor accurate (50).

Information on accumulation in aquatic species is limited to mostly PFOA and PFOS, and most BAF values generated are from field studies. A summary of data regarding field based BAFs and BMFs is given in Table 2.3. Martin *et al.* provided one of the first comprehensive sets of data regarding the laboratory investigations of bioconcentration (47) and dietary accumulation (BAF) (51) using a suite of PFCAs and PFSAAs in aquatic organisms. Results of these studies indicated that for PFCAs and PFSAAs with perfluoroalkyl chain length less than 7 and 6 respectively, there was insignificant bioaccumulation and bioconcentration in rainbow trout tissues, however both factors increased with increasing chain length, and are greater for sulfonates than carboxylates with equivalent perfluoroalkyl chain length, thus showing that the functional group is also an affecting factor in PFAA bioaccumulation. PFCA BCFs increased by a factor of 8 for each additional perfluoro-alkyl moiety for PFCAs C₈-C₁₂, ranging from 4.0 to 23,000 based on wet weight concentrations.

Liu *et al.* (2011) (50) however reported the results of a laboratory investigation into the bioaccumulation of PFAAs in green mussels, where longer chain PFCAs and PFSAAs were shown to have the highest bioaccumulation potential, particularly PFDA and PFOS. PFOA was the least accumulative compound with higher depuration rates; PFDA and PFOS were found to have much slower depuration rates. Liu *et al.* also reported a concentration dependence on BAF, with the lower dose (1 µg/L vs 10 µg/L)

resulting in larger BAFs, the sensitivity to which increased with perfluoroalkyl chain length. The authors conclude that the bioaccumulation of PFAAs followed an adsorption model with both uptake and elimination first order reactions implying that continuous exposure of PFAAs at a certain level would be able to maintain an observed tissue concentration. Jeon *et al.* (52) also investigated PFAA bioaccumulation in the Pacific Oyster, reporting PFUnA to be the most bioaccumulative PFCA, with PFOA and PFDA completely eliminated after 28 days of depuration, while PFOS and PFUnA still remaining in the tissues. This is consistent with PFOS being one of the most detected PFAA in wildlife, (with mean concentrations up to 1900 ng/g w/w (53) even though environmental concentrations of PFOA and PFOS are comparable (50).

The US EPA considers BCF values <1000 to indicate that a substance is not bioaccumulative, BCF values, as reported by Martin *et al.* (47) are higher in liver or blood samples compared with whole body homogenates, consistent with the tendency of PFAAs to preferentially partition to proteins. For regulatory assessments, whole body estimates are considered more appropriate and with less-bias (54) (55). For larger animals, whole-body burden estimates, based on tissue concentrations and mass distribution, were recommended (54).

Table 2.3: Brief summary of BCF, BAF and BMF values reported for aquatic organisms.

Sample	Type	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTTrA	PFTeA	PFHxS	PFOS	Ref.
BCF = $C_{\text{organism}}/C_{\text{water}}$ (L/kg) Laboratory based											
Rainbow trout	Whole body	4		450	2700	18000		23000	9.6	1100	(47)
Rainbow trout	Blood	27		2700	11000	4000		30000	76	4300	(47)
Leopard frog	Whole body									83.1	(56)
Wild turtle	Serum	0.8-15.8								500-3800	(57)
Zebra mussels	Whole body									1000	(58)
Bass	Liver	184								8850	(59)
Mussels	Whole body	15	144	838						378	(50)
BAF = $C_{\text{organism}}/C_{\text{water}}$ (L/kg) Field Based											
Fish/following AFF spill	Whole body	7.6	112	2344	2951				71	1995	(60)
Fish/following AFF spill	Liver	25	427	5495	3388				74	12589	(60)
Fish/following AFFF spill	Liver									6300-125000	(61)
Lake trout	Whole body	1580	3980	7940					501	12600	(62)
BMF = $C_{\text{predator}}/C_{\text{prey}}$											
Lake trout/Alewife	Whole body	0.6	5.3	4.4	6.4	1.9	3.1	>2.6		3.7	(63)
Dolphin/all fish	Whole body	1.8-13	1.4-24	2.4-8.8	1.9-3.9	0.1-1.8			3.3-14	0.8-4	(54)
Fish/zooplankton	Whole body					2.5-156			9.1-10	12-35	(54)
Arctic cod/zooplankton				0.5		0.3					(64)
Seal/Arctic cod	Blood			1.4	3.1	0.8				7.0	(64)
Beluga/cod	Blood	0.9	12.9	55	229	3.2				179	(65)
Beluga/herring	Blood	1.3	5.8	87	353	7.9				276	(65)

The biomagnification of PFAAs was investigated by Martin *et al.* (2004) (63) in various organisms through a food web in Lake Ontario. PFOS was again the predominant PFAA at all trophic levels, followed by long chain (8-15 carbon) PFCAs, and bioaccumulation was indeed occurring at the top of the food web. Additionally, the authors reported that the highest PFAA concentrations were determined in pelagic feeding organisms, indicating that sediment were the major source of PFAAs to the food chain rather than water. Houde *et al.* (54) also investigated biomagnification of a range of PFAAs in the food web of the bottlenose dolphin, PFOS and long-chain PFCAs were also shown to biomagnify in the bottlenose dolphin food web, with concentrations increasing by several factors between predator and prey. The authors also conclude that the local WWTP effluents were the likely source of PFAAs to the region,

Predatory animals, such as fish eating mink and bald eagles, have body PFOS concentrations greater than the concentrations in their diets, suggesting PFOS can bioaccumulate to higher trophic levels of the food chain (20). Mink from the Midwestern US contained significant concentrations of PFOS in their livers with an estimated biomagnification factor of 22, following a laboratory experiment where the mink were fed fish from Saginaw bay, Michigan (66). Trophic magnification of PFOS was also observed through a food web in the Canadian Arctic, where stable isotopes of nitrogen were analyzed to assess relative trophic levels, with a trophic magnification factor of 3.1, whereas PFOA did not biomagnify through the foodweb, though was found to biomagnify between specific individual feeding pairs, such as between cod and beluga whales (37 and references therein).

2.6 Routes of Human Exposure

PFAAs have been detected in human blood worldwide, with reported concentrations ranging from 3.0 - 6.4 µg/L PFOA, 0.4 - 2.6 µg/L PFNA, 0.2 - 0.5 µg/L PFDA, 0.1 - 0.7 µg/L PFUnA, 0.02 - 0.14 µg/L PFDoA, and 13 - 56 µg/L PFOS (67, 68, 69, 70, 71, 72). According to the US EPA, PFC biomonitoring studies have found that the median blood serum level of PFOS in US women decreased from 24 µg/L (1999-2000) to 9 µg/L in 2007-2008, and PFOA levels decreased from 5 to 3 µg/L in the same time period, reflective of the regulatory and industrial stewardship actions to restrict production and use of >C₈ PFAAs, however serum levels of PFNA increased significantly from 0.5 to 1.2 µg/L, and levels of PFHxS have remained constant (6, 73). PFOA, having 7 perfluoroalkyl carbons, shows less of a tendency to be retained in biota, however this does not appear to be the case in human, as PFOA has been found to readily accumulate in human serum (74).

The routes by which humans have become contaminated by PFAAs are poorly understood, but are likely to include three major pathways: ingestion of food and water, from inhalation of indoor air and dust, and human to human transference via placental transfer or through lactation, as reviewed recently by Stahl *et al.* (75). PFAAs have been detected in fish, meat, dairy products and in plants. The ingestion of contaminated foodstuff and drinking water is considered to be the major pathway of human exposure (76, 77, 78). Transfer of PFCs from contaminated soil has been shown by (79), which may be of concern in regions where sewage amendments are used to fertilize crops. In Germany, consumption of fresh and salt water fish was reported to account for approximately 90% of the total dietary exposure (reported in (75) which is likely related

to the potential for biomagnification of PFAAs in the food chain. Of all foodstuffs examined, none besides fish have been found to reach a level of contamination high enough to result in reaching the tolerable daily intake (TDI) concentration for PFOS (150 ng/kg) or PFOA (1,500 ng/kg) in populations that are heavy fish consumers (80). Drinking water has a relatively smaller role in PFAA exposure assessments unless the water is contaminated by a specific source or event, as reported in the case of the PFAA contaminated Ohio River in West Virginia and Ohio, where drinking water wells as far away as 20 miles were contaminated by releases from an industrial source (81).

Exposure may also be derived from contact of food with non-food materials, PFOA is used in the production of non-stick cookware and may be present in residual amounts, though Begley *et al.* (2005) (82) reported that nonstick cookware contributed less to PFAA exposure than coated paper products such as microwave popcorn bags, where PFOA concentrations in popcorn measured up to 300 µg/kg. PFCs have been detected in house dust, with PFBS, PFHxS, PFOS, PFHxA and PFOA detected in the majority of samples (83) (84). These likely originate from textile products such as carpets and upholstery, and may be of concern in regards to the exposure of PFAAs to infants and small children as they are more often in contact with the floor with gathered dust, the carpet sources, and because of their frequent hand to mouth behavior (85, 86). Finally, PFAAs have been detected in umbilical cord samples (87, 88) and in human milk samples (70) suggesting exposure of PFAAs could occur to the developing fetus or nursing infant.

2.7 Toxicity and Human Health Risk

2.7.1 Toxicity

In addition to concerns regarding the persistent and bioaccumulative nature of perfluoro compounds, there is also mounting evidence concerning the toxicity, including evidence that PFAAs are endocrine disrupting compounds (89). Over the past 15 years there have been many animal studies conducted regarding the toxicity effects of perfluorochemicals, with results suggesting that PFAAs are capable of acting as hepato- (90) neuronal- (91) immuno- (92) and developmental (93) toxins.

Toxicokinetics

The uptake of PFAAs has been shown to occur by oral, inhalation or dermal exposure; oral uptake of PFOA and PFOS results in a rapid uptake and 93-95% assimilation within 24 hours in male rats, dermal exposure was on the other hand found to be relatively weaker (75). Once inside the body, PFAAs preferentially bind to proteins, specifically, serum albumin (90-99%) (94), and lipoproteins and fatty acid binding protein in the liver (95). There is no known metabolism of PFCAs and PFSA in mammals; however Butt *et al.* (96) detailed a biotransformation pathway of FTOHs in rainbow trout and determined that PFCAs were generated. Excretion of PFAAs is the only route of elimination. In rats PFOS has an elimination half-life of 90 days, but PFOA has a much shorter half-life which varies with gender; 2-4 hours in female rates and 4-6 days in males (97). A sex hormone regulated active excretory mechanism has been described in rats, named the organic anion transporter (OAT), which removes PFOA from the blood via the proximal cells in the kidneys (98). As the half-lives of PFAAs have

been reported to be much longer in humans (PFOS 5.4 years, PFOA 3.8 years, and PFHxS 8.5 years), considerations must be taken with the use of animal models in determining PFAA risk for humans (75).

Toxicodynamics

Based on the results of animal experiments, the acute toxicity of PFAAs is considered modest, and not likely to be of concern at most commonly reported environmental levels (75). The liver is considered to be the primary organ of toxic effects. Hepatotoxicity via a peroxisome proliferation mechanism has been reported in rats fed with low (<0.01%) levels of PFOA and PFDA (90), and in rats exposed to subchronic levels of PFOS (99). Researchers have recently begun to ascertain if the toxicity effects observed in laboratory animals are also an outcome from wildlife exposed to environmentally relevant concentrations. Hoff *et al.* (100) reported PFOS levels in the muscle of carp resulted in a disruption of hepatocyte membrane integrity. Positive correlations have also been observed between PFOS liver concentrations in wood-mice (101) and in carp and eel (102), with biochemical and physiological indicators of liver toxicity.

A recent review by DeWitt *et al.* (92) concerning the immunotoxicity of PFCs finds mounting evidence of immune effects, including alterations of inflammatory responses, cytokine production and adaptive or innate immune responses, seen in laboratory animals occurring at serum concentrations below or within the reported range of serum levels reported for exposed wildlife and humans. PFOA is a known developmental toxicant that has been shown to produce long lasting effects in

reproductive tissues and with metabolic programming in mice and other animal models, and can also alter steroid hormone production, as recently reviewed by White *et al.* (103). A brief summary of reported toxic effects of PFAAs is given in Table 2.4.

Table 2.4: A brief summary of reported toxic effects for PFAAs in laboratory experiments, as reported in the recent review by (75).

PFC	Study	Species	Target organ/Effect
PFOS PFOA	Subacute/ subchronic	Rats	Body weight ↓ liver mass↑, liver vacuolization Peroxisome proliferation, liver mass↑ hepatocellular necrosis
PFOS	Chronic/ carcinogenic	Rats	Hepatotoxicity; adenomas of liver+thyroid+breast
PFOS PFOA	Reproduct/ develop	Rats mice	↓ Increase body weight; ↓ #live births+viability of progeny ↑ Liver weight+fetal reabsorption; ↓progeny weight gain
PFOS PFOA	Neurotoxicity	Chicken Mice	Impaired cognitive performance in hatched chicks ↓ adaptability; hyperactivity
PFSAs PFCAs	Endocrine	Rats Trout	Effects on thyroid hormones Weak xenoestrogens (VTG)
PFOS PFOA	Immunotox	Rats Mice	Altered inflammatory response, cytokine production, weight of lymphatic organs, antibody synthesis
PFOS PFOA	Epidemiol	Humans	Correlations with: ↓birth weight, risk of ADHD, fertility disorders Carcinogenic- questionable

While the toxicity of PFOS and PFOA have been extensively reported in animal studies, the research on the potential health effects for humans is generally inconclusive, but does suggest a number of important health effects such as decreased sperm count (104) lower birth weight and size (105) and thyroid disease (106). In view of the widespread occurrence and possible for negative health effects, guidelines on chronic exposure are being developed by the EPA, although little has been done so far for PFC compounds other than PFOA and PFOS (6).

2.7.2 Ecotoxicity

It is apparent that due to their unique physicochemical characteristics, PFAAs tend to persist in surface waters, therefore the potential adverse effects on aquatic organisms should be considered with priority. Studies have reported on the toxicity of PFAAs with algae aquatic plants, invertebrates, zooplankton, amphibians and fish. Boudreau, *et al.* (107) reported a no observable effect concentration (NOEC) for PFOS of 5.3 and 8.2 mg/L in two species of green algae using cell density as the end point. NOECs were also determined for *Daphnia magna* (water flea) of 12 and 7 mg/L of PFOS for reproduction, and 12 mg/L for survival and growth in the 21 day test (as summarized in (8)). Ji *et al.* (108) reported a 21-day reproduction NOEC for *D.magna* of 1.25 mg/L for PFOS and 12.5 mg/L for PFOA. The lowest observable effect concentrations (LOEC) for PFOA and PFOS on zooplankton community were reported to be 30-70 mg/L and 10-30 respectively (109). Ankley *et al.* (56) reported reduced growth and time to metamorphosis in the 3 mg/L exposure of PFOS to the northern leopard frog (*Rana pipiens*). In another study, Ankley *et al.* (110) exposed sexually mature fathead minnow (*Pimephales promelas*) to a range of PFOS concentrations

(0.03 - 1 mg/L). The 1 mg/L concentration was lethal to adults following 2 weeks of exposure. While there were no significant effects on the developing minnows exposed to 24 days of up to 0.3 mg/L PFOS, at 0.3 mg/L decreased aromatase activity and elevated testosterone was measured in adult males, and histopathological alterations were observed on the ovaries of the females.

While the aim of most toxicological studies is to determine lethal or sub-lethal concentrations, a few studies have attempted to ascertain whether the concentrations of PFAAs measured either in the biota or in the surrounding environment are able to promote the same toxic effects observed in laboratory studies. Using the reported PFOS concentrations by Giesy & Kannan (20) of 300 µg/kg in carp muscle and 2.6 mg/kg in eagle blood serum, Hoff *et al.* (100) were able to induce disruption of carp hepatocyte membrane integrity. In another study, Hoff *et al.* (111) reported a significant positive correlation between measured PFOS concentrations in the liver of both carp and eel (0.011 – 9 mg/kg), and the serum alanine aminotransferase activity (a bio-indicator for liver damage). Finally, plasma concentrations of PFOS and PFOA were also seen in the loggerhead sea turtle to correlate with indicators of liver damage or reduced immune function respectively (112). A brief summary of additional reported ecotoxicity effects is given in Table 2.5.

2.8 Occurrence in and Sources to the Aquatic Environment

PFC pollution in the aquatic environment has been documented in freshwater lakes (113) and river systems (114, 115, 116), in coastal regions (117, 118, 119), in seas (120) and in open ocean surface waters (40, 41, 121). Additionally, the extent of

PFC presence has been documented in samples of air (46), snow (122) and ice (123) in the Arctic, and in the surface waters of the Southern Ocean (124, 125). The highest PFC concentrations are generally found in areas that are the closest to direct industrial emission sources, with aquatic concentrations reported in ranges from 1-1000s of ng/L. (126). Concentrations typically observed in open ocean surface waters are 3 orders of magnitude lower, ranging from 10-100 pg/L (6, 127). A brief summary of reported concentrations of PFAAs in aquatic systems is given in Table 2.6.

Table 2.5: Brief summary of reported toxic effects in aquatic organisms:

Species:	PFC Concentrations:	Toxicity Effects:	Reference
Loggerhead sea turtle, Southeast US Atlantic coast.	Plasma PFOS: 1.4 – 96.8 ng/mL	Positively correlated with indicator of liver damage.	(112)
	Plasma PFOA: up to 0.993 ng/mL	Negatively correlated with indicator of immune function.	
Daphnia	PFOS 100 µg/L	Survival, emergence and growth inhibited by 50%	(128)
Male minnow	PFOA: 3 mg/L for 28 d	Estrogenic effects (hepatic VTG and testis-ova gonads)	(129)
Zebra fish	Chronic effects: PFOS: 50 – 250 µg/L	Liver alterations. Hepatic VTG gene expression up regulated. Low maternal [PFOS] exposure could result in offspring deformation/mortality	(130)

The occurrence of PFAAs locally has been reported by Sinclair *et al.* (59) following an extensive study of nine major water bodies (freshwater lakes and rivers) in

New York State. The most commonly detected PFAAs were PFOS, PFOA and perfluorohexane sulfonic acid (PFHxS), detected in surface waters in concentrations of 0.8-1090 ng/L, 10-173 ng/L and 0.5-8.5 ng/L respectively. Sinclair *et al.* additionally investigated the concentration of detected PFAAs in fish tissues, finding that the average PFOS concentrations of PFOS in fish samples was 8850 times greater than those in surface water, highlighting the significance of dietary fish in PFOS accumulation in the food chain. The highest concentrations measured in this study were in Lake Onondaga, which the authors note is a superfund site that is influenced by several industrial sources located in the region, as well as the Metropolitan Syracuse WWTP discharge which makes up approximately 20% of the lake's annual inflow. The highest reported PFAA concentration in water body are in those regions either impacted directly by fluorochemical industries, as measured in the Tennessee River by Hansen *et al.* (131) or those that utilize fluorochemicals such as airports (39). High PFAA concentrations are also measured following accidental releases or spills, as reported by Moody *et al.* (61) in Etobicoke Creek following a release of fire retardant foam from the nearby Toronto airport, where PFHxS, PFOS and PFOA were measured in surface waters in concentrations up to 49.6, 2210 and 2270 µg/L respectively. These concentrations are of particular concern as they exceed the predicted no effect concentrations (PNECs), as established by the UK Environment Agency for protecting wildlife, of 25 µg/L in freshwater and 2.5 µg/L in saltwater, for PFOS (132).

Wastewater treatment plants are known point-sources of PFAAs to the aquatic environment with concentrations reported, in ng/L to µg/L ranges, in the US (133, 134, 135, 136, 137), Europe (114, 138, 139, 140, 141) and in Asia (38, 142, 143, 144, 145,

146, 147). Some examples of the concentrations reported are given in Table 2.7; for a more extensive review on the occurrence of PFAA in WWTPs, see (148). The most extensively studied PFAAs are PFOA and PFOS, with the occurrence of other PFAAs being more limited. In the US, maximum PFOS and PFOA concentrations reported were 400 ng/L and 184 ng/L respectively (148). Studies examining the mass balance and fate of PFAAs throughout the wastewater treatment process have determined that PFAA are not significantly removed by secondary biological treatment (135, 141, 142, 149). Additionally, concentrations are also reported to have increased in the treated final effluent compared to the influent (134, 136, 141). While biodegradation of the PFCAs and PFSAAs does not seem to occur, the transformation of precursor compounds to PFAAs during the wastewater treatment process has been suggested (134, 149), as well as implied by the results of several laboratory studies (148).

While the substantial mass loading of PFAAs in WWTPs is discharged in the final effluent, sorption to sludge is also an important mechanism from PFAA removal in the treatment processes, specifically for longer-chain compounds. A number of papers have published data regarding the sorption of PFOS and PFOA to different types of sludge in laboratory batch experiments, including the study by Zhou *et al.* (150), determining partitioning ($\log K_d$) values for PFOS and PFOA of 2.3 - 3.6 and 2.2 - 2.5 respectively using activated sludge. Very few studies have reported on the partitioning of other PFAAs with sewage sludge; Arvaniti *et al.* (151) calculates K_{oc} values from PFOA, PFDA, PFUnA and PFOS in three different types of sewage sludge, reporting highest $\log K_{oc}$ values obtained with secondary sludge, in the range of 2.96 – 4.69 for the PFAAs studied. Guerra *et al.* (149) reported $\log K_d$ values for sludge PFOS (3.73),

PFDA (3.68), PFNA, (3.25), PFOA (2.49) and PFHxA (1.93). Arvaniti *et al.* (151) further examined the mass balances of a suite of PFAAs in a typical WWTP utilizing calculated partitioning coefficients, concluding that PFAAs with < 10 carbons are detected mainly in treated effluent, whereas longer-chain PFAAs will be removed (>60%) via primary and secondary sludge treatment.

The presence of long-chain PFAAs in sewage sludge becomes a further issue due to the use of sewage derived biosolids used for soil amendments. Application of contaminated biosolids from a local WWTP in Decatur, Alabama, was reported by Lindstrom *et al.* (152) to result in the PFAA contamination of surface and well water samples in concentrations exceeding the US EPA's Provisional Health Advisory level for PFOA in drinking water of 400 ng/L. Another secondary contamination event in the US from the use of WWTP effluent was reported by Konwich, *et al.* (35), where a land application system (where treated effluent is sprayed to the landscape) in Dalton, GA, resulted in PFAA concentrations measured in the Altamaha River (up to 1150 ng/L PFOA, 318 ng/L PFOS, 369 ng/L PFNA and 113 ng/L PFDA) among the highest measured at a non-spill or direct-release location. Urban run-off has also been reported to be a significant non-point source of PFCAs to the aquatic environment (38, 153, 154). Kim and Kannan (153) reported PFAA contamination was higher in surface runoff, especially from heavily trafficked roadways and parking lots, than in rainfall, and suggested potential sources to be automotive windshield washer fluids, polishes and fuel additives. Some reported values of PFAA concentrations in point and non-point sources to the aquatic environment are given in Table 2.7.

Table 2.6: A brief survey of PFAA concentrations (ng/L) reported in lakes, rivers, coastal seas and open ocean waters.

Sample	Location	PFAA	Reported concentration	Reference
Lake water	Albany, NY	PFHpA	1.15-12.7	(153)
		PFOA	3.27-15.8	
		PFNA	Nd-3.51	
		PFDA	0.25-3.58	
		PFUnA	Nd-1.45	
		PFDoA	Nd-<LOQ	
		PFHxS	<LOQ-4.05	
		PFOS	Nd-9.30	
		PFDS	Nd-0.34	
Lake water	Taihu Lake, China	PFHpA	Nd-18.4	(116)
		PFOA	10.6-36.7	
		PFDoA	Nd-3.2	
		PFHxS	Nd-6.5	
		PFOS	3.6-394	
River water	Tennessee River, TN,	PFOS	16.8-144	(131)
		PFOA	<LOQ-598	
River water	Upper Mississippi River, IL, IA, MN, MO, WI	PFBA	Nd-458	(126)
		PFPA	Nd-31.5	
		PFHxA	Nd-53.4	
		PFHpA	Nd-90.2	
		PFOA	Nd-125	
		PFNA	Nd-72.9	
		PFDA	Nd-42.0	
		PFUnA	Nd-29.1	
		PFDoA	Nd-24.7	
		PFBS	Nd-84.1	
		PFHxS	Nd-169	
		PFOS	Nd-245	
		PFDS	nd	
River water	Toronto, Canada	PFHxA	4.0-14	(155)
		PFHpA	1.2-4.5	
		PFOA	2.2-7.9	
		PFNA	0.8-2.5	
		PFDA	0.33-1.6	
		PFUnA	0.07-0.44	
		PFDoA	0.026-0.48	
		PFTTrA	Nd-0.086	
		PFTeA	Nd-0.13	
		PFBS	0.27-1.7	
		PFHxS	2.1-6.5	
		PFOS	2.1-6.5	
Coastal sea	Antarctica	PFBA	<LOQ-0.08	(156)

		PFPA	0.03-0.08	
		PFHxA	0.11-0.36	
		PFHpA	<LOQ-0.03	
		PFOA	0.08-15.1	
		PFUnA	<LOQ-0.04	
		PFDoA	<LOQ-0.04	
		PFTTrA	<LOQ-0.23	
		PFTeA	0.01-0.09	
		PFDS	8.3-8.6	
Coastal Sea	East to South China Sea	PFPA	<LOQ-0.44	(157)
		PFBS	<LOQ-0.94	
		PFHxA	<LOQ-0.30	
		PFHpA	<LOQ-0.42	
		PFOA	<LOQ-1.54	
		PFOS	<LOQ-0.07	
		PFNA	<LOQ-0.04	
		PFTTrA	<LOQ=0.03	
Open ocean	Arctic Ocean	PFBA	<LOQ-0.36	(157)
		PFPA	0.03-0.26	
		PFHxA	<LOQ-0.03	
		PFHpA	<LOQ-0.16	
		PFOA	<LOQ-0.07	
		PFNA	<LOQ-0.05	
		PFUnA	<LOQ-0.02	
		PFBS	<LOQ-0.08	
		PFOS	<LOQ-0.05	
		PFDS	<LOQ-0.01	
Open ocean	Northwest Pacific Ocean	PFBA	<LOQ-0.18	(121)
		PFPA	<LOQ-0.15	
		PFHpA	<LOQ-0.28	
		PFOA	<LOQ-0.10	
		PFNA	<LOQ-0.07	
		PFDA	<LOQ-0.04	
		PFUnA	<LOQ-0.03	
		PFBS	<LOQ-0.10	
		PFOS	<LOQ-0.06	
		PFDS	<LOQ-0.02	

Table 2.7: A brief survey of reported PFAA concentrations (ng/L) in point and non-point sources to the aquatic environment.

Sample	Location	PFAA	Reported concentration	Reference
Non-point sources:				
Air/particle	Albany, NY	PFHpA	<LOQ-0.81	(153)
		PFOA	0.76-4.19	
		PFNA	<LOQ-0.40	
		PFDA	0.13-0.49	
		PFUnA	Nd	
		PFDoA	<LOQ-0.38	
		PFHxS	<LOQ	
		PFOS	0.35-1.16	
		PFDS	<LOQ-0.18	
Snow	Albany, NY	PFHpA	<LOQ-1.61	(153)
		PFOA	<LOQ-19.6	
		PFNA	<LOQ-4.94	
		PFDA	Nd-1.37	
		PFUnA	Nd=1.08	
		PFDoA	Nd-0.41	
		PFHxS	Nd-0.35	
		PFOS	<LOQ-1.93	
		PFDS	Nd-<LOQ	
Snow	Toronto, Canada	PFHxA	0.06-4.7	(155)
		PFHpA	0.04-2.0	
		PFOA	0.05-3.7	
		PFNA	0.07-0.99	
		PFDA	0.01-0.49	
		PFUnA	0.002-0.25	
		PFDoA	Nd-0.12	
		PFTTrA	Nd-0.044	
		PFHxS	0.001-0.19	
Snow	Antarctica	PFOS	Nd-0.87	(156)
		PFBA	0.076-1.112	
		PFPA	<LOQ=0.203	
		PFHxA	0.14-0.68	
		PFHpA	<LOQ	
		PFBS	<LOQ-0.017	
		PFHpS	<LOQ-0.053	
		PFOS	0.017-0.020	
		PFDS	0.018	
Precipitation	Northeastern US, southeastern Canada	PFBA	<LOQ-23	(158)
		PFPA	<LOQ-39	
		PFHxA	<LOQ-42	
		PFHpA	<LOQ-31	
		PFOA	<LOQ-89	

		PFNA	<LOQ-77	
		PFDA	<LOQ-1.1	
		PFUnA	<LOQ-3.7	
		PFDoA	<LOQ-5.2	
Precipitation	Albany, NY	PFHpA	<LOQ-2.32	(153)
		PFOA	<LOQ-7.27	
		PFNA	<LOQ-3.48	
		PFDA	Nd-1.14	
		PFUnA	<LOQ-0.86	
		PFDoA	<LOQ-0.71	
		PFHxS	Nd-0.36	
		PFOS	<LOQ-1.51	
		PFDS	Nd-0.41	
Surface run-off	Albany, NY	PFHpA	<LOQ-6.44	(153)
		PFOA	0.51-29.3	
		PFNA	<LOQ-5.90	
		PFDA	Nd-8.39	
		PFUnA	Nd-1.99	
		PFDoA	Nd-1.60	
		PFHxS	Nd-13.5	
		PFOS	<LOQ-14.6	
		PFDS	Nd	
Surface run-off	Antarctica	PFBA	1.4306	(156)
		PFPA	0.038	
		PFHxA	0.064	
		PFHpA	0.175	
		PFBS	<LOQ	
		PFHpS	<LOQ	
		PFOS	0045	
		PFDS	0.018	
Point Sources:				
WWTP effluent	Developed areas, China	PFBA	Nd-19.6	(159)
		PFPA	Nd-5.7	
		PFHxA	Nd-55.3	
		PFHpA	Nd-7.4	
		PFOA	Nd-106.6	
		PFNA	Nd-7.4	
		PFDA	Nd-8.3	
		PFUnA	Nd-2.8	
		PFDoA	Nd-0.9	
		PFBS	Nd-30.6	
		PFOS	Nd-67.3	
WWTP effluent	Greece	PFPA	<LOQ-209.4	(141)
		PFHxA	<LOQ-3.9	
		PFHpA	<LOQ-11.5	
		PFOA	<LOQ-34.0	
		PFNA	<LOQ-10.3	
		PFDA	<LOQ-15.9	

		PUnA	<LOQ-27.5	
		PFD _o A	<LOQ-33.9	
		PFT _r A	<LOQ-46.6	
		PFT _e A	<LOQ-62.4	
		PFH _x S	<LOQ-5.8	
		PFH _p S	<LOQ-8.6	
		PFOS	<LOQ-21.0	
		PFDS	<LOQ-35.1	
WWTP effluent	Korea	PFH _x A	1.1-14.8	(142)
		PFH _p A	<LOQ-16.1	
		PFOA	3.4-49.2	
		PFNA	<LOQ-15.8	
		PFDA	Nd-4.2	
		PFH _x S	Nd-10.5	
		PFH _p S	Nd-0.8	
		PFOS	0.9-4.6	
WWTP effluent	Germany	PFOA	20-73	(140)
		PFOS	60-390	
WWTP effluent	Hong Kong	PFH _x A	0.7-1.2	(143)
		PFOA	4.1	
		PFNA	0.6	
		PFBS	1.3-1.5	
		PFOS	19-28.8	

2.9 Occurrence in Sediments and Partitioning Behavior

Longer-chain PFAAs do show a tendency to distribute between solid and aqueous phases in sorption studies, as the hydrophobic properties of the fluoro-carbon chain become more prominent with increased chain length, with reported controlling factors including sediment organic matter content (25), black carbon, iron oxide and clay content (29). PFAA partitioning has been described as a predominantly entropy driven process (160), however solution chemistry has also been shown to effect partitioning, such as calcium ion (Ca^{2+}) concentration and pH, signifying that electrostatic interactions also play a role (25).

PFAAs have been detected in sediments from freshwater, coastal and marine environments worldwide (Table 2.8). Concentrations are generally reported in the low (~1 ng/g) to mid-level (~100 ng/g dry weight) ranges. Typically sediment PFAA concentrations are highest in the vicinity of local urban and industrial emission sources, particularly in river and fresh water sediments in the proximity of WWTP effluent discharge. Becker *et al.* (161) determined sediment concentrations of PFOA and PFOS downstream from a WWTP effluent discharge zone to be 3 and 40 times higher in the sediment relative to the river water. As with all other environmental matrices, PFOA and PFOS are the most commonly investigated PFAAs. Pan and You (162) reported one of the highest sediment PFOS concentrations of 536.7 ng/g, in the river mouth of the Yangtze River, China, an area, the authors report, which is heavily impacted with human activities; PFOS had previously only been reported at this concentration extent in sewage sludge (163). The highest reported sediment concentrations of PFOA (76.9 ng/g) were measured in the sediments near an industrial park in Laizhou Bay, China (Table 2.8) (34). PFOS was also measured in the sediment of the wastewater canal of Pančevo (Serbia) industrial area in concentrations of up to 5.7 ng/g, by Beškoski *et al.* (164); in this study a suite of PFAAs was investigated, with PFHxS, PFDS, PFHxA and PFOA also detected in the sediments in low concentrations ranges of <LOQ to 0.23 (PFHxS), 0.29 (PFDS), 0.17 (PFHxA) and 0.13 ng/g (PFOA).

Coastal marine sediments are typically less contaminated, reflecting the increasing distance from the emission sources and/or dilution as the higher concentration PFAA river waters mix with the lesser contaminated sea waters. While sorption to sediments appears to be an important fate for PFAAs in the proximity of

emission discharge zones, the solid-water distributions of PFAAs may additionally influenced by solution chemistry, as they travel from fresh riverine waters to the saline environments of the estuaries. Salinity effects were recently reported by Pan and You (162) where PFOS sediment sorption increased with increasing salinity at the mouth of the Yangtze River; with log K_d values increasing from ~ 0.6 (0.14 ‰) to 4.6 (3.31‰). These observations suggest that coastal estuaries will be an important trap and potential sink for PFAAs transported by rivers from emissions sources towards the ocean.

Table 2.8: A brief survey of reported PFAA concentrations (ng/g dry weight) in marine and riverine sediments.

Sample	Location	PFAA	Reported concentration	Reference
Riverine	Yangtze River, China	PFOS	72.9-536.7	(162)
Riverine	Roter Main, Germany downstream of WWTP,	PFOA PFOS	0.085 \pm 0.060 0.280 \pm 0.120	(161)
Riverine	Netherlands	PFOA PFNA PFBS PFOS	0.3-6.3 0.1-5.7 <0.1-13 0.5-8.7	(165)
Riverine	Orge River, France	PFHxA PFHpA PFNA PFDA PFUnA PFDoA PFTTrA PFTTeA PFHxS PFHpS PFOS PFDS	0.06 \pm 0.01 0.03 \pm 0.01 0.05 \pm 0.01 0.30 \pm 0.02 0.29 \pm 0.01 1.7 \pm 0.0 0.30 \pm 0.01 0.86 \pm 0.03 0.10 \pm 0.02 0.03 \pm 0.01 4.3 \pm 0.3 0.12 \pm 0.01	(166)
Riverine	Laizhou Bay, China	PFBS PFHxS	0.02-0.60 0.04-0.52	(34)

		PFHpS	0.10-0.91	
		PFOS	0.02-1.6	
		PFDS	0.07	
		PFPA	0-2.5	
		PFHxA	0.03-1.9	
		PFOA	0.04-76.9	
		PFNA	0.01-0.64	
		PFDA	0.01-1.21	
		PFUnA	0.01-0.86	
		PFDoA	0.01-0.81	
		PFTTrA	0.01-0.41	
		PFTeA	0.01-0.26	
Riverine	Georgia, US	PFHxA	<LOQ-0.40	(167)
		PFHpA	<LOQ-0.39	
		PFOA	0.06-1.97	
		PFNA	0.03-0.68	
		PFDA	0.03-4.66	
		PFUnA	<LOQ-3.80	
		PFDoA	<LOQ-4.64	
		PFTTrA	0.07-0.98	
		PFTeA	0.05-1.67	
		PFBS	<LOQ-0.22	
		PFHxS	<LOQ-0.17	
		PFOS	<LOQ-20.18	
Coastal	Laizhou Bay, China	PFBS	0.02-0.04	(34)
		PFHxS	0.02-0.32	
		PFHpS	0.09-0.17	
		PFOS	0.03-0.06	
		PFDS	0.09-0.12	
		PFPA	0.01-0.02	
		PFOA	0.07-1.8	
		PFNA	0.01-0.08	
		PFDA	0.01-0.06	
		PFUnA	0.01-0.07	
		PFTTrA	0.01-0.03	
Coastal	San Francisco Bay	PFHxS	Nd-0.072	(163)
		PFOS	Nd-3.76	
		PFDS	Nd-2.70	
		PFOA	Nd-0.625	
		PFNA	Nd-0.237	
		PFDA	Nd-1.11	
		PFUnA	Nd-0.396	
		PFDoA	Nd-0.584	
		PFTTrA	Nd-0.435	
Coastal	Tokyo Bay, Japan	PFHxS	0.046-0.056	(168)
		PFOS	0.096-0.128	
		PFOA	<LOQ-0.007	
		PFNA	0.009-0.014	

PFDA	0.006-0.008
PFUnA	0.064-0.066
PFD _o A	0.018
PFT _e A	0.004-0.007

2.9.1 The Role of Suspended Particulate Matter in Biogeochemical Cycling

Sedimentation of SPM at the mouth of a river serves as a sink, removing contaminants from the water column, but resolubilization and mobilization may occur as partitioning of contaminants is influenced by salinity changes in the mixing zone of estuaries. In evaluation of aquatic distributions and fate of pollutants, distinguishing between the freely dissolved and particulate bound forms and the factors that influence this distribution is of importance, particularly for pollutant transport modelling and risk assessment studies. The partitioning coefficient, bioavailability of chemical components and assimilation efficiencies are key parameters that require definition in order to perform accurate biogeochemical modelling (169). The biogeochemical dynamics of PFAAs remains scarcely documented, with even fewer field studies reporting the partitioning of PFAAs between the aqueous and SPM phases in riverine (116, 166) or estuarine (170) systems.

Results from the field studies indicate that the PFAA sorbed fraction correlates positively with SPM concentrations (171), and is influenced by organic carbon content (170), as is reported for laboratory studies investigating dissolved-sediment partitioning dynamics (25). Of particular interest with the aqueous-SPM distribution dynamic is the observation that the organic carbon partition coefficient ($\log K_{oc}$) for SPM is >1 order of magnitude greater than the K_{oc} for sediments in estuarine field studies (170, 171) which

indicate that particulate size difference may influence the sorption capacity. The sorptive characteristics of particles are generally a function of particle size or surface area of exposed sorbable matter, as well as chemical composition; permanently suspended particulates are reported to have greater surface area per unit mass, with more reactive coating and greater density of bacterial coverage (169). The role of SPM is of particular importance due to the nature of these particulates and their capacity to sorb hydrophobic organic micropollutants, and the pivotal role that the SPM plays as a link between the water column, bed sediment and food chain for associated contaminants (169).

2.10 Conclusions

PFAAs are globally distributed, persistent pollutants that have been shown to bioaccumulate and biomagnify in the environment, particularly in aquatic organisms such as fish and fish eating predators such as minks and dolphins. PFAAs have been shown to elicit toxic effects in animal studies, and bio-indicators of these specific toxicities, including liver damage and endocrine disruption, have been measured in aquatic organisms in the field with significant correlations to measured PFAA blood, liver or body burden levels. While the PBT nature of PFAAs has been recognized, and significant legislation and industrial stewardship actions have taken place to mitigate the continued environmental contamination of PFAAs, continuing emissions from an increasing PFC production industry in Asia, in addition to no current restrictions of the production and emission of precursor compounds, mean that continued scientific scrutiny in this matter is very much still warranted.

Many questions still remain regarding the origins and distribution of PFAAs in the aqueous environment (7, 172). There is a dearth of environmental contamination data, as well as a lack of partitioning data at environmentally relevant levels (173). Therefore it is clear that data regarding regional marine PFAA inputs would be invaluable in contributing to the scientific understanding of the global PFAA transport budgets, as well as contribute to the field based observations of biogeochemical behavior and fate of PFAAs in the coastal marine environment.

Field based observations of solid-water distributions of PFAAs are necessary, particularly of the longer chained, more bioaccumulative ($C \geq 8$) PFAAs as laboratory derived partitioning constants, which are generally accepted for pollutant transport modelling, are suspected to be lower than those obtained in the field, which can lead to an over estimation of the aqueous phase PFAA concentrations and underestimation of sediment contamination; and thus consequently an underestimation of the potential risk to local benthic ecosystems. Studies have reported that PFAAs in sediments are readily bioavailable to benthic invertebrates (174), can adversely affect benthic organisms (175), and that sediment bound PFAAs were the major source of PFAAs to a food web in Lake Ontario (63). In addition, partitioning to suspended particulates is hypothesized to be of greater magnitude than predicted by laboratory batch sorption experiments, as has been reported in several field studies. SPM provides a crucial link for the movement of contaminants to the food chain. As consumption of fish and sea food has been shown to be the major route of exposure of PFAAs to humans, investigating the role SPM plays in PFAA biogeochemical cycles is crucial. Particular interest in this dissertation research is directed towards better understanding the dynamics between water column and SPM

phase PFAA distributions, by comparison of riverine, estuarine and effluent SPM. The sorption of PFAAs to SPM present in wastewater effluent (efSPM) may be of particular interest due to the high (approximately 50%) protein composition (176). Sewage derived particulate matter is a mixture of organic detritus and microorganisms such as bacteria and algae, which is a high quality food source to aquatic consumers, thus could potentially be an important vector in the transfer of sorbed contaminants to the food chain.

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Occurrence and Partitioning Behavior of Perfluoroalkyl Acids in Wastewater Effluent Discharging into the Long Island Sound Tributaries.

Abstract

Perfluorinated compounds (PFCs) have been extensively used for their unique properties in numerous industrial and commercial applications since the 1950's; properties which include a chemical stability that has also proved resistant to biotic and abiotic environmental degradation, leading to their ubiquitous presence in practically every environmental compartment and distribution worldwide. Wastewater treatment plants (WWTPs) are considered a major point source for the entry of PFCs to the aqueous environment. This study reports on the presence of 14 perfluoroalkyl acids (PFAAs) (C_4 - C_{12} carboxylic acids (PFCAs) and C_4 - C_{10} sulfonic acids (PFSAs)) in wastewater effluents discharging into the CT shoreline region of the Long Island Sound (LIS) and tributaries. A survey of the final effluents obtained from 12 wastewater treatment plants (WWTPs), conducted in the spring and

summer of 2012 confirmed PFAAs present in concentrations ranges consistent with literature values. The bioaccumulative 8 carbon chain species, PFOA and PFOS, were found to be the most prevalent PFAAs in the effluent streams, but shorter chained carboxylic acid PFAAs were also detected in similar concentrations, likely reflective of an industry/EPA stewardship program move towards replacing the longer chained PFCs with shorter chained PFCs with lesser bioaccumulation potential. Partitioning constants derived for water and suspended particulate matter in the effluent stream (efSPM) were found to be one to two orders of magnitude greater (Log K_{oc} of 4.62 ± 0.32 and 5.21 ± 0.40 for PFOA and PFOS respectively) than those previously reported for sludge, with log K_{oc} values increasing with perfluoroalkyl chain length by ~ 0.4 log units per CF_2 moiety. As efSPM is considered a high quality food source to aquatic biota, it is plausible that efSPM could act as an efficient vector of PFAAs into the food chain.

LIS effluent loading estimates were obtained from the discharge monitoring report pollutant loading tool (US EPA) with data obtained from the US National Estuaries Program. The total average daily flow from all treated effluent ranged from 2.98×10^9 L day⁻¹ to 3.92×10^9 L day⁻¹ between 2011 and 2014; an average daily input of 3.3×10^9 . Based on an average 3.3 trillion liters of treated wastewater entering the LIS watershed daily, and the minimum and maximum concentrations measured, the annual Σ PFAA (sum of all PFAA congeners detected) mass flow into the LIS from WWTPs was estimated at 140 – 1,460 g/day or 50 - 530 kg/year. The results in this study represent the first report on PFAA concentrations in effluent discharging into the LIS watershed from CT WWTPs.

3.1 Introduction

Effluent water from waste water treatment plants (WWTPs) discharging into local waterways is a known point source of nutrients and trace contaminants. Many of these chemicals are classified as Emerging Contaminants, as the consequences of their presence in the environment are yet to be fully understood and regulated. Perfluoroalkyl compounds are such an example of commercially useful anthropogenic compounds which have gained widespread use due to their distinctive physical and chemical characteristics. PFAAs comprises of a fully fluorinated carbon chain attached to a hydrophilic head group. Their unique characteristics are imparted by the fluorinated chain region; a function of the strength of the carbon-fluorine bond, and of the non-bonding electrons of the fluorine atoms, which provide a compact repellent electron shield that make PFCs resistant to attack (1). Resulting characteristics include water and oil repellency, chemical and thermal stability, inertness, and surface active properties in both aqueous and solvent systems that are incomparable (2). PFCs have been used commercially since the 1950's in applications such as stain repellants, in products that include textiles and paper packaging, as surfactants and dispersants in formulations such as paints, cosmetics, lubricants and firefighting foam, and in the production of fluoropolymers such as Teflon® (3). The features have made PFCs virtually irreplaceable in their many applications also impart the characteristics of persistent organic pollutants (POPs); they are environmentally persistent, bioaccumulative and toxic (PBT) (2).

Although there are numerous poly- and per-fluorinated alkyl compounds currently produced, the most widely reported in the literature are the perfluorocarboxylic acids

(PFCAs) and the perfluorosulfonic acids (PFSAs), in particular the 8-carbon chained species perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Unlike most POPs which tend to accumulate in lipids, PFCs are proteophilic, thus tend to accumulate in blood, liver and kidneys (4, 5, 6). Bioconcentration and bioaccumulation is a function of chain length, with PFSAs being more bioaccumulative than PFCAs, and shorter chained (seven or less fluorinated carbons) PFCAs are not considered bioaccumulative, even though they are environmentally persistent and are in detectable levels in wildlife (7). In humans, PFAAs have been detected in blood serum of people living in the industrialized parts of the world in the ng/mL range (8). Reported toxic effects of PFAAs include hepatotoxicity (9), immunotoxicity (10), and neurodevelopmental toxicity (11, 12).

PFAAs are distributed globally, and have been detected in almost all environmental compartments, including air, water, wildlife, food and humans, and like classical POPs, PFAAs have been shown to undergo polar distillation (3) leading to their detection in remote locations, including Arctic wildlife (11, 13) and open-ocean surface waters (14, 15). A recent study of contaminants in Arctic polar bears reported PFOS in concentrations surpassing that of other POPs such as polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) (16). Mounting evidence of the PBT nature of PFAAs led to the voluntary discontinuation of PFOS and related compounds by the principal manufacturer, 3M, working with the US EPA between 2000 and 2002, as well as inclusion of these compounds to the Stockholm Convention on POPs in 2009, thereby restricting manufacture and use. However PFAA emissions continue to be of global concern as production in developing regions increased to fulfill

industrial demands (3, 17) as well as the continued production and use of numerous new PFCs, many of which are less soluble and more volatile than the perfluorinated acids, and are capable of long-range atmospheric transport. These include fluorotelomer alcohols (FTOHs) and perfluoroalkyl sulfonamides (FASAs), compounds which have also been shown to degrade to the persistent PFCAs and PFSAAs in both the atmosphere (18, 19) as well as under aerobic conditions, in, for example, activated sewage sludge (14, 20). As FTOHs have an atmospheric half life of 20 days (21), atmospheric transport of volatile precursors, and subsequent degradation by atmospheric oxidation and deposition has been determined to be a major route in the global distribution and presence in remote locations of PFCAs and PFSAAs (22). As these compounds possess anionic hydrophilic groups and are considerably soluble, they are also capable of being transported long distance by ocean currents (23). The relative contribution of each transport pathway is of intense research interest; therefore elucidating regional influences of PFCs to local marine environs and ultimately to the oceanic pathway is of great value.

The discharge of municipal wastewater from WWTPs is one of the principal point sources of PFAAs to the aquatic environment. Moreover, effluent mass flows of PFOA have been reported to be found 1.3- to 4.5-fold higher in the effluent compared to that in the influent (24, 25, 26), suggesting that formation of these final persistent degradation products from precursor compounds is occurring during the waste water treatment process (26, 27). Clearly WWTPs are a major point source for the introduction of PFCs into the local aqueous environment (27, 28, 29). Around the Connecticut (CT) and New York (NY) shorelines there are 44 WWTPs that discharge over 1 billion gallons of

treated wastewater effluent into the Long Island Sound (LIS) every day (30). The LIS is an economically important urban sea, recently valued at providing \$17 - \$36.6 billion annually in natural capital assets (31). The LIS is utilized recreationally, industrially and commercially, with a population of approximately 16 million people living and working on its shores, thus understanding any potential impacts from anthropogenic activities is of critical importance to this region. In addition, the understanding of PFAA biogeochemistry in aquatic systems is still limited by the lack of field data describing distributional behavior in different systems. The partitioning of persistent organic pollutants describes the tendency of a compound to be found in, or to move to or from, an environmental phase or compartment. Understanding the distributions of persistent, bioaccumulative and toxic pollutants, and obtaining reliable expressions for partitioning processes is key for environmental modeling, to be able to predict the impact of human activities on the environment and to determine the potential for human exposure (32).

Studies have reported on the partitioning of PFAAs between dissolved and solid phases with wastewater sludge (26, 33, 34) and riverine (35, 36) or estuarine sediments (37, 38). Few studies have investigated the partitioning of PFAAs between the dissolved and suspended particulate matter (SPM) phase in river (39) or estuarine (38) systems and only one study has investigated wastewater effluent particulates (40). Some results have indicated that PFAA partitioning is slightly greater to SPM compared to bed sediments (36, 38). In this study partitioning to effluent suspended particulate matter (efSPM) in the final effluent is of particular interest, as efSPM has been shown to be a high quality food source preferentially assimilated by benthic biota and therefore could be an effective vector for the transport of pollutants into the food chain (41).

This aims of this study were to determine the occurrence, range of mass flows and to investigate partitioning behaviors of PFCAs and PFSAAs in treated wastewater effluent discharging into the shoreline LIS from several CT WWTPs, in order to provide information on the potential of effluent derived PFAA loadings to the LIS. Though the presence of PFAAs has been reported in a growing number of aquatic systems, this is the first report on the presence of the compounds from WWTPs located in the CT shoreline region.

3.2 Experimental

3.2.1 Chemicals and equipment

PFAA analytical standards were purchased from Waters Corporation (Milford, MA) and included PFAA calibration standards (11 PFAAs (C₄-C₁₀) and 5 PFSAAs (C₄, C₆-C₈, C₁₀), PFAA mass labeled recovery standards (Perfluorohexanoic acid (1,2-¹³C₂), Perfluoro-n-[1,2-¹³C₂]decanoic acid and Perfluorohexanesulfonic acid (¹⁸O₂)) and PFAA internal standards (1,2,3,4-¹³C₄PFOA and 1,2,3,4-¹³C₄PFOS). A complete description of each target analyte is given in the Chapter 2, Table 2.1. Methanol (Optima LC/MS grade) ammonium hydroxide and ammonium acetate were purchased from Fisher Scientific. All equipment used was pre-cleaned by baking at 450 °C for 4 hours minimum (glass) or thoroughly washed and rinsed with MilliQ and methanol (for non-glass components). All SPE tubing, valves and adapters were sonicated in methanol for a minimum of three 10 minute rinses.

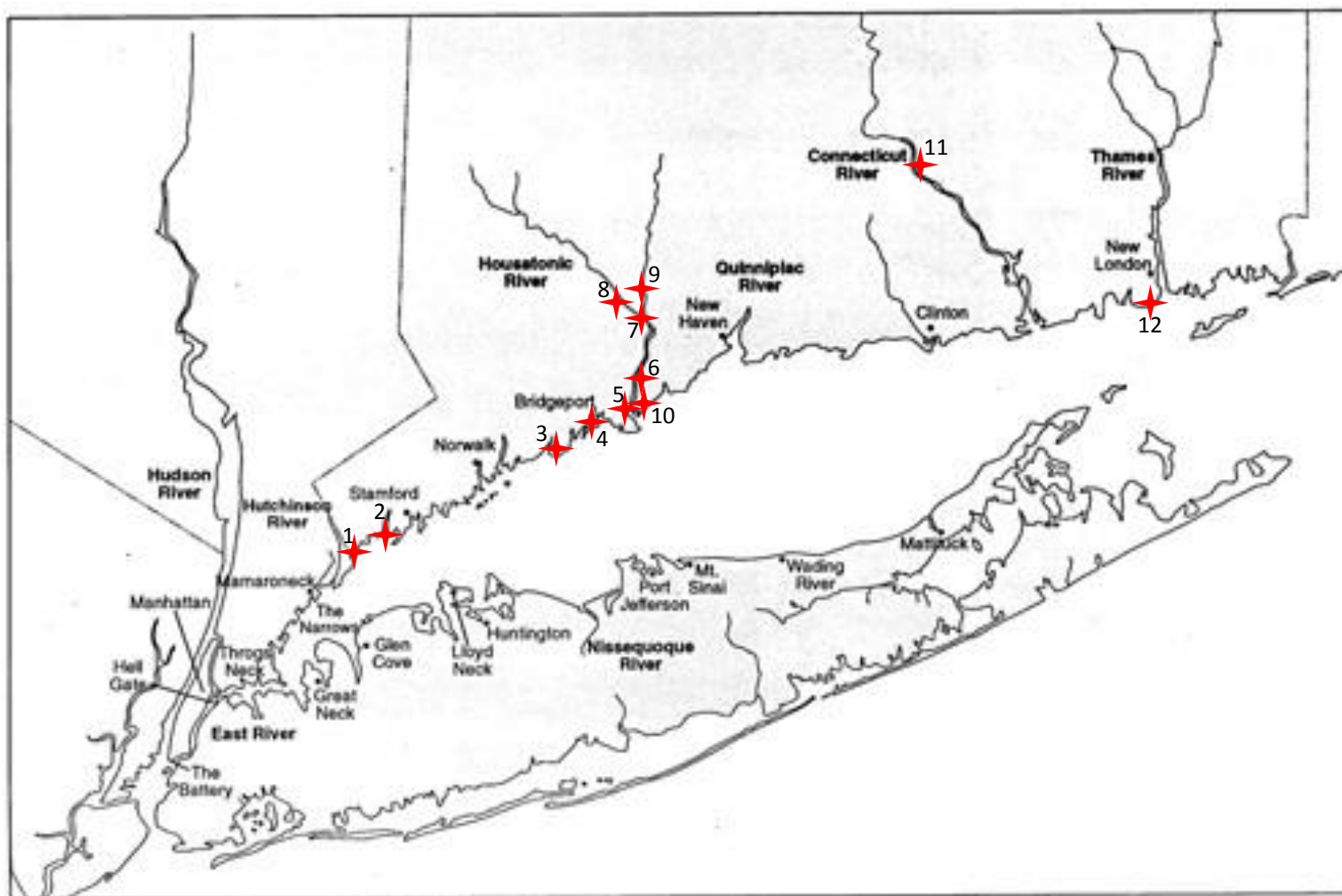


Figure 3.1: Map of sampled WWTP locations (red star markers) around the CT shoreline and along two of the largest rivers flowing to the Long Island Sound, the Housatonic and the Connecticut Rivers (Map obtained from LongIslandSoundStudy.net). 1- Greenwich, 2- Stamford, 3- Fairfield, 4- Bridgeport Westside, 5- Stratford, 6- Milford Housatonic, 7- Shelton, 8- Derby, 9- Ansonia, 10- Milford Beaverbrook, 11- Mattabassett, 12- New London.

3.2.2 Sampling sites and collection

Of the twelve WWTPs sampled, six are located along the CT shoreline and discharge into harbor areas along the LIS (Greenwich, Stamford, Fairfield, Bridgeport Westside and New London). Of the remaining six, five are located further up the Housatonic (Derby, Shelton, Ansonia, Milford Housatonic and Stratford), one discharges into a local salt marsh near the mouth of the Housatonic River (Milford Beaverbrook) and one is located on the Connecticut River (Mattabassett District) (Figure 3.1); the Housatonic and Connecticut Rivers are the two largest rivers supplying fresh water to the Sound. Three plants were relatively high flow rate facilities (16-21 MGD), five were medium flow (6-10 MGD) and four were smaller facilities with flow rates between 1.5 - 2.2 MGD. Summary information on the WWTPs, collection dates and water quality parameters, is given in Tables 3.1 and 3.2. Grab or flow proportional 24 hour composite samples of final effluent were collected in either polypropylene (PP) or high density polyethylene (HDPE) carboys at each of the waste water facilities. Samples were stored at 4°C during and following collection, and extracted within 24 hours.

3.2.3 Sample extraction

1L samples of effluent were filtered through 0.45 µm nominal pore size polypropylene (PP) membranes (previously rinsed with 3x methanol) and the filtrate collected in pre-cleaned 1L PP bottles.

Suspended particulate matter (SPM) extraction: The PP filters were placed into pre-cleaned 15 mL PP centrifuge tubes, 5 ng of PFAA recovery standard mix was spiked onto each filter, and 12 mL methanol added. Tubes were refrigerated for a 24 hour extraction, followed by sonication for 1 hour. The extract was then decanted into a

second PP centrifuge tube and stored at 4°C, while the filters were extracted a second time in methanol for 24 hours at 4°C followed by sonication. Both methanol extracts were combined and reduced under a gentle stream of ultra-pure nitrogen to a final volume of 0.5 mL. The final extracts were then cleaned according to a method previously reported (42, 43). 50 mg of ENVI-carb (Supelco) was weighed into a 1.5 mL PP centrifuge tube, and triple rinsed by vortexing in methanol. The extract was added to the ENVI-carb, vortexed for 10 seconds, centrifuged, and then transferred to a clean 1.5 mL PP vial.

Aqueous phase extraction: Two 250 mL samples of filtrate were placed into pre-cleaned 500mL PP bottles and spiked with 10ng of the PFAA recovery standard mix for solid phase extraction (SPE) using a method previously described (27). Oasis HLB Plus (225mg) SPE cartridges (Waters Corporation, Milford, MA) were mounted on a Miniprep® vacuum manifold, however all poly(tetrafluoroethylene) (PTFE) materials were removed and replaced by PP counterparts to avoid any potential fluoropolymer contamination. Extraction cartridges were pre-cleaned with two 10 mL aliquots of methanol then conditioned with 5 mL of MilliQ water. Samples were extracted at a flow rate of approximately 50µL/second, washed with 10mL of 10% Methanol in MilliQ water and dried under vacuum for 20 minutes, then finally were eluted with two aliquots of 7 mL of methanol into a PP tube.

Table 3.1: Spring sampling; collection dates and water quality parameters.

Sampling Date	WWTP	Flow (MGD)	Sample type	Disinfection	% Industrial input ^a	Final effluent temp °C	pH	Receiving water body
3/23/12	Ansonia	1.83	24h composite	None	n/a	17.2	6.7	Naugatuck River
3/29/12	Bridgeport	21.5	Grab	Chlorine	0.5%	14.7	6.9	Black Rock Harbor
3/2/12	Derby	1.81	24h comp. + grab	Chlorine	12%	11.7	6.7	Housatonic River
5/4/12	Fairfield	8.67	24h composite	UV	0.1%	15.6	6.8	Long Island Sound
5/4/12	Greenwich	9.8	24h composite	UV	n/a	16.7	6.8	Greenwich Harbor
5/4/12	Mattabassett	16.9	24h composite	Chlorine	n/a	18.3	6.8	Connecticut River
3/2/12	Milford Housatonic	6.14	24h comp + grab	UV	n/a	14.0	6.2	Housatonic River
5/4/12	New London	6.65	Grab	Chlorine	0.2%	16.1	6.7	Thames River
3/29/12	Shelton	2.191	24h composite	None	2.3%	14.4	6.8	Housatonic River
5/4/12	Stamford	16.8	24h composite	UV	12%	18.1	7.0	Stamford Harbor
3/23/12	Stratford	6.3	24h composite	UV	20%	18.2	6.7	Housatonic River

MGD = Mega gallons per day

a = Average annual estimate. Information obtained from Dennis Grecci, CT DEEP (personal communication).

n/a=Information not available as per Dennis Grecci (CT DEEP).

Table 3.2: Summer sampling collection dates and water quality parameters.

WWTP	% Industrial input	Dates	Flow MGD	Sample type	Disinfection	Final effluent temp °C	pH	Receiving water body
Ansonia	n/a	7/17/12	1.3	24h composite	UV	23.3	6.8	Housatonic River
		7/23/12	1.4			23.3	6.6	
Derby	12%	7/17/12	1.4	24h composite	Chlorine	21.1	6.8	Housatonic River
		7/23/12	1.5			21.1	6.8	
Milford	n/a	7/17/12	4.9	24h composite	UV	24.2	6.7	Housatonic River
Housatonic		7/23/12	4.8			23.0	6.4	
Shelton	2.3%	7/17/12	1.9	24h composite	Chlorine	23.3	6.4	Housatonic River
		7/23/12	1.7			22.8	6.3	
Stratford	20%	7/17/12	6.0	24h composite	UV	25.0	6.9	Housatonic River
		7/23/12	5.1			24.6	6.8	
Milford	n/a	7/17/12	1.5	24h composite	UV	22.5	6.7	Housatonic River
Beaverbrook		7/23/12	1.5			22.3	6.7	

MGD = Mega gallons per day

a = Average annual estimate. Information obtained from Dennis Grecci, CT DEEP (personal communication).

n/a=Information not available as per Dennis Grecci (CT DEEP).

Elutes were concentrated under a gentle stream of ultra-high purity nitrogen to a final volume of 1 mL. For the summer sampling campaign, all extractions were performed as described with the exception of the SPE cartridge type; weak anion exchange (WAX) columns were used, washed with 5 mL of 25 mM sodium acetate buffer (pH 4) followed by 5 mL of MilliQ, then eluted with 10 mL 0.1% ammonium hydroxide in methanol. The WAX columns are reported to provide better clean up, help overcome matrix interferences and improve retention of the shortest target acids (<C6) (44). Prior to instrumental analysis, all extracts were filtered with a 0.2 μ m nylon syringe filter (pre-rinsed with methanol) into a 300 μ L PP autosampler vial with PP screw cap.

3.2.4 Instrumental analysis and quantitation

Samples were analyzed for 16 target PFAAs (11 PFCAs- C4-C14, and 5 PFSA- C_{4,6-8,10}) on a Waters Acquity ultra-performance liquid chromatography system with triple quadrupole tandem mass spectrometer (UPLC-MS/MS) at the Center for Environmental Science and Engineering (CESE) at the University of Connecticut. In order to remove any potential for contamination that has been reported for PFAA analysis due to the presence of fluoropolymers such as PTFE tubing (45) the UPLC was retrofitted using a PFC analysis kit from Waters Corporation, which is commercially available for this instrument. The retrofitting includes replacement of all PTFE tubing with polyether ether ketone (PEEK) materials, and a C18 hold-up column installed on the aqueous solvent line before the mixing chamber. Instrumental analysis parameters were as described previously (44). Briefly, 8 μ L aliquot of sample was injected onto an Acquity BEH C18 column (2.1 μ m x 50 mm; Waters Corp.) that was held at 50 °C. Analytes were eluted using a gradient mobile phase of 2mM ammonium acetate buffer in methanol at a flow

rate of 500 $\mu\text{L}/\text{min}$. Electrospray negative ionization (ESI) was used and the mass spectrometer was operated in multiple-reaction-monitoring (MRM) mode. Quantitation was performed using MassLynx software, with a linear $1/x$ weighted regression fit. Calibration curves were prepared from the methanolic standards in the range of 1-500 ng/mL and the instrumental detection limit was determined using a 3:1 signal to noise (S/N) ratio of the lowest concentration methanolic standard. UPLC run parameters and MRM transitions monitored are listed in Appendix (Tables A2 and A3).

3.2.5 Quality control

Pre-cleaned PP sample bottles containing MilliQ were used as field blanks to evaluate contamination during sample transport and storage; reagent blanks were used to evaluate extraction contamination. Additional pre-cleaned sample bottles containing MilliQ were spiked with 5 ng (absolute mass) PFAA mixture or with a PFAA QC solution mix (4.3–25 ng) to evaluate analyte loss and extraction performance. Blank PP filters were also extracted; using PFAA spiked (5 ng) filters to evaluate extraction performance, and unspiked filters, to evaluate contamination throughout the extraction procedure. An ENVI-carb clean up blank was also performed, as well as a nylon-needle filter blank, to ascertain that these additional steps could yield any contamination.

The limit of quantitation can vary from one analysis to another as it can dependent upon background. Since effluent, riverine and estuarine waters constitute different potential background effects, reporting limits were set as follows; limit of detection (LOD) and limit of quantitation (LOQ) were set at signal to noise ratio (S/N) >3 and $S/N >10$ respectively for each individual target analyte peak in each sample run. Data was therefore only deemed reportable for each individual peak with a S/N ratio

>10. Reported concentrations were corrected for potential ion suppression or enhancement using isotopically labeled recovery standards (Appendix Table A3). Instrument detection limits of each target PFAA were estimated by extrapolation from the smallest calibration standard (Appendix Table A4). The SPE extraction method was initially validated by spiking replicate samples of ultrapure (MilliQ) water and samples of filtered effluent (Appendix Figures A1 and A2). Recoveries of PFBA were very low (<40%) and no peaks were detected above the MDL for PFTrA and PFTeA. Recoveries for the spiked MilliQ and filtered effluent were within acceptable ranges for the remaining PFCAs, and optimal from C₅-C₁₁, ranging from 74% - 98%. PFSA recoveries were also within acceptable ranges for both MilliQ and effluent samples, with recoveries decreasing with increasing perfluoroalkyl chain length, ranging from 97%-63% in effluent and 111% to 54% in MilliQ for C₄-C₁₀ PFBS-PFDS (Appendix Figures A1 and A2). Adjusting recoveries using recovery standards resulted in increasing the recovery percentage and, for the majority of the samples, decreasing the standard deviation (Appendix Figures A1, A2 and A3).

Method performance was additionally evaluated by extracting replicate samples of MilliQ water (aqueous phase) and PP filter membranes (SPM phase) spiked with PFAA mix with each extraction set (Appendix Table A5 and Figures A3 and A4). For PP extractions, recoveries were within acceptable ranges for PFCAs C₄-C₁₂ and for all PFSAs, ranging from 53%-85% without adjusting with recovery standards, or 66%-100% after adjustment with recovery standards (Figures A3 and A4). During the spring sampling periods, poor SPE recoveries (no peaks with S/N ratio > 10) were obtained for the compounds PFBA, PFTrA and PFTeA, and of PFTrA and PFTeA during the

summer, therefore no data is reported for these compounds in the dissolved phase samples.

No PFAA target analytes were detected in any of the field, reagent, PP filter, or ENVI-carb clean up blanks above the instrument detection limit with the exception of PFOA detected in one of MilliQ lab blanks at a concentration below MDL, and a large PFHxS signal seen in all blanks and QC samples indicating a specific error with the analysis of this particular target compound for samples obtained during the spring sampling season. This error was corrected for the analysis of the summer sampling campaign. The use of WAX columns during the summer resulted in better recoveries of the smallest PFAA compound, PFBA, increasing average recovery from 44% to 116% (Appendix Table A5). Additionally, PFTTrA and PFTTeA were detected with peaks above the MDL. Details regarding the amount of recovery standards detected for each sampling survey are given in the Appendix Table A6.

3.2.6 Elemental analysis

Samples of final effluent (60 - 150mL) were filtered through pre-combusted GF/F glass fiber filters (GFF). SPM collected on the GFFs was lyophilized and analyzed for total carbon and nitrogen on the Costech 4010 elemental analyzer. Additional SPM samples collected during the summer survey were acidified overnight prior to organic carbon analysis.

3.3 Results and discussion

3.3.1 Concentrations and composition profiles of PFAAs in final effluent

For studies investigating the potential environmental mass loadings of pollutants from wastewater effluent discharge, collection of a one-time 24 hour flow proportional composite sample is generally preferred to a one time random grab sample, as the composite sample provides information on average conditions, and generally regarded to have less variability than samples collected at constant volume-constant time intervals (48). However, Schaeffer *et al.* using model simulations based on waste stream parameters determined that random grabs serve as well as composite samples for monitoring purposes (49). In this study, 24 hour flow proportioned composite samples were obtained from 9 of the WWTFs under investigation, however at two locations, grab samples were obtained due to composite sample unavailability. The potential variability between grab and the composite samples was investigated in a preliminary study on two WWTPs (Derby and Milford Housatonic) where both grab and composite samples were collected. Grab samples were obtained within 1-2 hours of the completion of the composite sample collection program, and all samples were processed simultaneously. No significant difference was observed in the concentrations of PFAAs detected in grab compared to 24 hour composite samples taken at these two WWTPs, though PFNA was only detected in the composite sample from plant Milford Housatonic.

PFAAs have been detected in WWTP effluent worldwide. Due to the exceptional stability of the fluorinated molecule, as well as solubility and reduced tendency to sorb to waste sludge, the conventional treatments utilized in wastewater remediation are not

considered effective in removal of these compounds (26, 46, 47). Overall, 13 of the 16 target PFAAs were detected in effluent water, including PFCAs C₄-C₁₂ and PFSA C_{4,6-8,10}. PFAA concentration averages and ranges for the 11 WWTPs sampled in the spring and the 6 facilities sampled in the summer of 2012 are shown in Figure 3.2; individual concentration values are summarized in Appendix Tables A7 and A8.

PFOA was the predominant PFCA (detected in 100% of samples) contaminant in aqueous samples obtained spring 2012, with average (range) concentrations of 33.3 (6.3 - 64) ng/L, however PFPA and PFHxA were also detected at similar concentrations (23.4 (6.1 - 46.4) ng/L and 28.5 (6.2 - 47) ng/L respectively). The predominant PFSA detected was PFOS (observed in 80% of samples) at an average (range) concentration of 11 (<MDL - 29.3) ng/L. The relative concentrations of the PFAA congeners vary across the published data, though the most commonly detected in the highest concentrations (and also most often studied) are PFOA and PFOS. However it is apparent in the more recent publications, that there is an increase in the occurrence and concentration of the shorter chained PFAAs such as PFPA, PFHxA, PFBS and PFHxS (34, 50). This trend towards shorter PFAA species detected is likely a reflection of recent stewardship programs restricting the uses and applications of the longer chain (C>7) perfluoralkyl compounds.

The concentrations of PFAAs observed in the CT WWTPs are generally similar in magnitude to those reported in locations in Greece (34), Germany (51), Denmark (52), Hong Kong (53), and in Korean domestic municipal waste, but lower than concentrations reported for Korean industrial or mixed industrial-domestic wastewaters (24). PFAA concentrations are also comparable to those reported in WWTPs in the

USA; in Iowa, PFOS and PFOA concentrations reported as 26 and 22 ng/L respectively (54), in Georgia (PFOA 6.7-102 ng/L and PFOS 1.8-13 ng/L) (55), and slightly lower, for PFOS, than those reported in Oregon (PFOA 2.5-97 ng/L, PFOS 1.1-130 ng/L) (55, 56) and for PFOA in Kentucky (PFOA 122-183 ng/L, PFOS 8-28 ng/L) (55). However, the PFAA concentrations reported in this study are considerably lower than PFAA concentrations reported in Taiwan (29), Thailand (47), Japan (57), and those determined locally in New York State (58). A brief summary of reported concentration values including other PFAA congeners detected in final effluent are given for comparison (Table 3.3).

Typically waste streams with higher percentages of industrial effluent see higher concentrations of PFAAs, particularly PFOA and PFOS (24, 50). PFOA was detected in relatively higher concentrations in Bridgeport, Stamford and Stratford, which comprise an estimated 0.5, 12 and 20% of industrial sourced input, based on CT Department of Energy and the Environment (Tables 3.1 and 3.2). Concentrations of PFOS were 2-3 times greater in Fairfield and Stamford, consistent with greater industrial input (12%) to both plants. The ratio of shorter chained PFAAs (PFPA and PFHxA to PFOA, and PFHxS to PFOS) was greater for WWTPs New London, Shelton, Stamford and Stratford, also consistent with the presence of industrial discharge to these plants, reflecting the shift in industrial use practices towards shorter chained PFAAs; however an increased presence of shorter chains PFAAs was not reflected in Bridgeport, Derby and Fairfield, which have known industrial inputs which may be a function of the type of industrial discharge to these sites. Ansonia, Stamford and Stratford had the highest Σ PFAA concentrations (202 ng/L, 230 ng/L and 198 ng/L respectively). Both Stamford

and Stratford both have higher known industrial influent compositions relative to the other WWTPs, with the exception of Derby. Derby, it should be noted, is one of the smaller facilities, with a daily flow rate of 1.9 MGD compared to 16.2 and 6.3 MGD for Stamford and Stratford.

Although the information regarding industrial inputs to a number of the WWTPs was not available, because either the town did not report it or they have no way of estimating, additional investigation into Ansonia determined over 70 listings of either pre-treatment or general permits issued by the DEEP Materials Management and Compliance Assurance (CT DEEP Dennis Grecci- personal communication) which could signify a substantial industrial component to the WWTP, consistent with the elevated PFAA concentrations detected. As the industrial inputs to each of the WWTPs included in this survey is unknown, no further conclusions or observations can be made regarding the influence of industrial inputs and PFAA concentrations. Daily Σ PFAA discharge from each WWTP and their composition profiles along with the total daily flow for each WWTP sampled in the spring survey are shown in Figure 3.3.

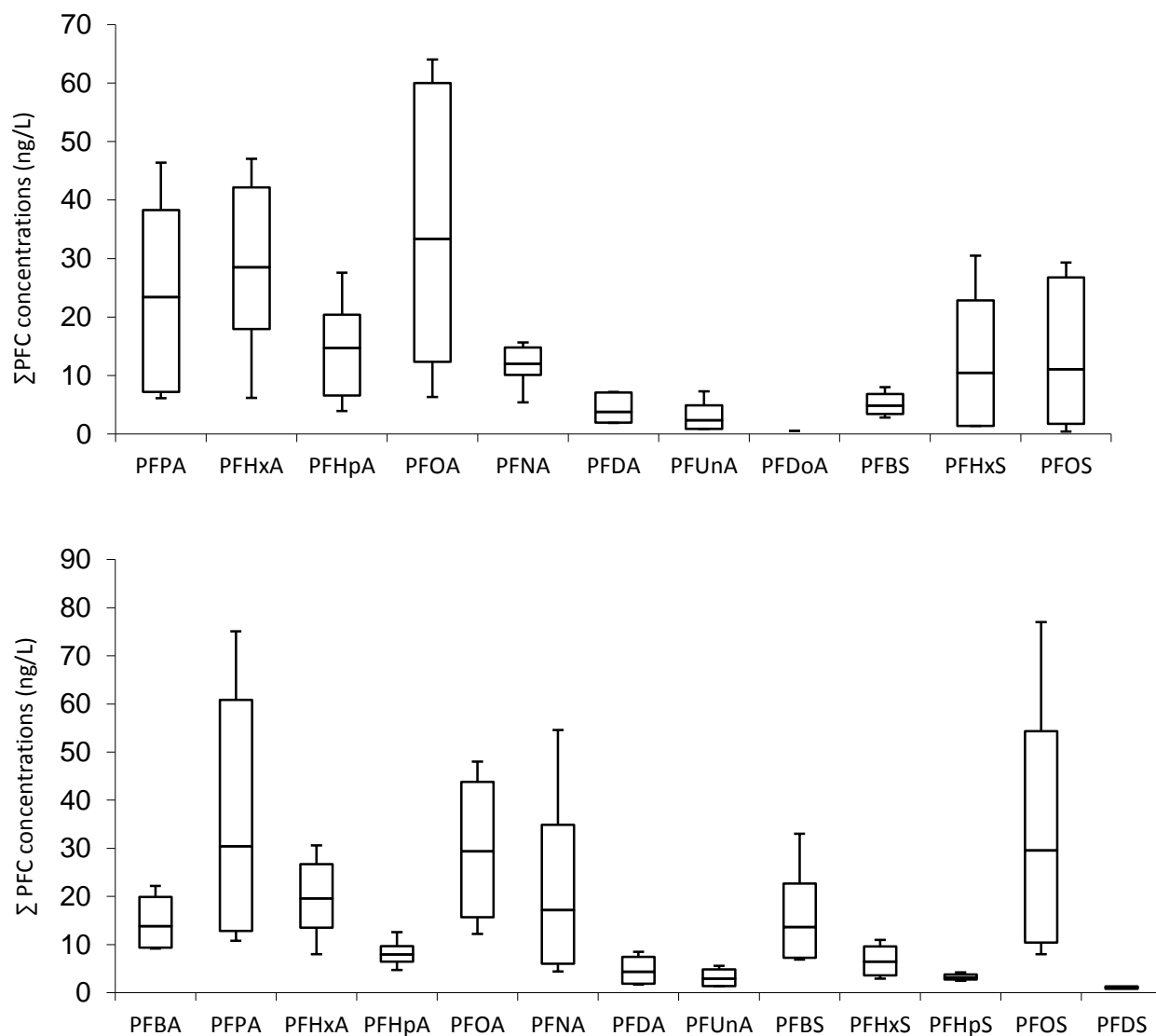


Figure 3.2: Box plots showing mean, 10th and 90th percentile, and range of ΣPFAA concentrations (dissolved plus SPM fractions) detected above MDL in final effluent from (top) 11 CT WWTPs, spring 2012 and in the effluent of the 6 WWTPs located along the Housatonic River, summer 2012 (bottom). In the spring survey, PFDoA was detected only once, PFBA was not detected due to poor recoveries, PFHpS and PFDS were not detected above MDL. In summer, PFDoA was not detected above MDL.

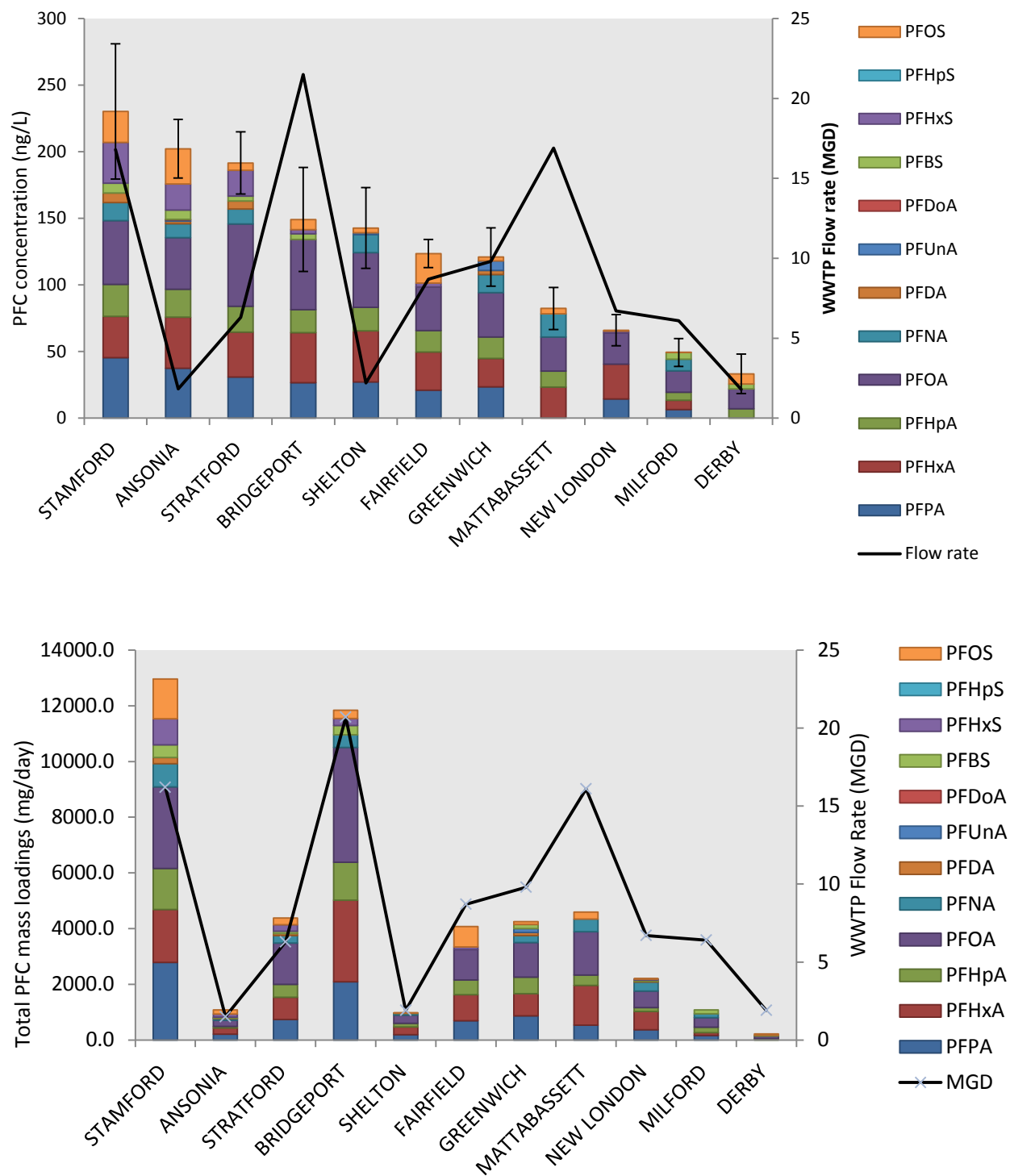


Figure 3.3: (Top) PFAA concentrations measured in final effluent (Error bars show range). (Bottom) Daily mass loadings (in mg/day) from each of the 11 WWTPs sampled, spring 2012.

Table 3.3: Average PFAA concentrations (min-max, both seasons) of PFAAs detected in this study, with a brief summary of recently reported effluent PFAA concentrations (ng/L) for comparison. Nd=not determined/not reported.

Location of WWTPs	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTra	PFTeA	PFBS	PFHxS	PFHpS	PFOS	Ref.
LIS/CT shoreline	13.9 (<LOD-21.2)	25.9 (<LOD-72.5)	25.2 (<LOD-47.0)	12.2 (<LOD-27.6)	32 (6.3-64.0)	14.4 (<LOD-54.6)	3.9 (<LOD-8.5)	2.5 (<LOD-7.3)	0.5 (<LOD-0.5)	<LOD	<LOD	9.3 (<LOD-33.0)	8.5 (<LOD-30.5)	3.2 (<LOD-4.2)	20.3 (<LOD-77.0)	This study
Developed areas, China	0.9-19.6	0.6-18	0.8-70	0.05-7.4	2.6-106	0.3-7.1	0.05-8.3	0.05-2.8	0.05-0.9	Nd	Nd	0.05-30.6	Nd	Nd	0.05-67.3	(50)
Greece	Nd	3.1-209.4	<LOD-3.6	1.0-11.5	12.7-34	<LOD-10.3	<LOD-15.9	<LOD-27.5	<LOD-33.9	<LOD-46.6	<LOD-62.4	<LOD	<LOD-5.8	<LOD-8.6	5.2-21.0	(34)
Tianji, China	5-25	10-100	12-275	1-12	30-145	5-25	1-8	<LOQ-2	<LOQ-1	nd	nd	nd	<LOQ-40	Nd	2-15	(26)
Bavaria, Germany	Nd	Nd	Nd	Nd	20-73	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	106-336	(25)
Hsinchu, Taiwan	Nd	Nd	71.1-180.7	<0.1-14.5	19.3-480	1.4-22.6	<0.1-4.8	<0.1-2.8	Nd	Nd	Nd	2.6-960	6.3-2226.7	nd	162.7-5663.3	(29)
River Elbe, Germany	Nd	1.5 – 40.9	3.7-57.4	1.6-15.7	12.3-77.6	1.0-18.6	0.9-34.5	<0.004-8.8	<0.01-0.5	<0.02-0.4	Nd	1.8-25.9	0.8-2.1	<0.08-0.5	<0.06-82.2	(51)
Ontario, Canada	Nd	Nd	Nd	Nd	5.8-180	<LOQ-4.2	<LOQ-3.2	<LOQ-0.08	Nd	Nd	Nd	Nd	Nd	Nd	<LOQ-72	(60)
Glatt Valley, Switzerland	Nd	Nd	0.7-33	0.3-6.3	12-35	<LOQ-0.8	<LOQ-2.8	Nd	Nd	Nd	Nd	<LOQ-7.8	2.8-88	Nd	16-303	(46)
Kentucky, USA	Nd	Nd	Nd	Nd	122-183	2.4-9.5	0.64-7.9	<LOQ	<LOQ	Nd	Nd	Nd	6.3-9.5	Nd	8-28	(55)
Iowa City, Iowa, US	Nd	Nd	Nd	Nd	19.9-24.1	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	24-28	(54)
Georgia, USA	Nd	Nd	Nd	Nd	6.7-102	1.2-15	<LOQ-4	<LOQ	<LOQ	Nd	Nd	Nd	<LOQ-8.3	Nd	1.8-13	(55)
New York, USA	Nd	Nd	Nd	Nd	58-1,050	4-376	<LOQ-47	<LOQ-10	Nd	Nd	Nd	Nd	<LOQ-39	Nd	3-68	(27)
Oregon, USA	Nd	Nd	3.4-20	1.0-23	2.5-97	0.7-6.1	<LOQ-28	Nd	Nd	Nd	Nd	<LOQ-20	2.4-17	Nd	1.1-130	(55)
Pacific Northwest, USA	Nd	Nd	4.6-8.3	Nd	8.2-15	1.5-5.9	0.6-5.1	Nd	Nd	Nd	Nd	Nd	<LOQ-3.2	Nd	15-34	(56)

3.3.2 Estimated loading to the LIS

Perfluoroalkyl acids have been reported in nonpoint sources such as stormwater runoff. In a study monitoring stormwater runoff in the Twin Cities (Minneapolis and St. Paul, MN) Xiao *et al.* detected PFAAs in 100% of samples, and estimated a mass flux of 7.9 kg/year in runoff from residential areas (78). In Japan, Zushi *et al.* reported that a river received a higher load of PFAAs from stormwater runoff than from WWTPs (79). Kim and Kannan reported that surface runoff contributes to PFOA contamination in Lakes near Albany, NY (80). A question in considering the potential mass loadings to the LIS from WWTP point sources is whether the PFAA concentrations measured are reflective of normal sanitary sewer operations, or if there are contributions from storm water runoff. Of the WWTPs included in this study, Bridgeport Westside was the only facility with a combined sewer and storm water system (CSO). Mattabassett also received stormwater to the facility, but from only one out of the three residential regions served (personal communications with the WWTP superintendents).

The question of CSO facility compared to separate sanitary operations (SSO) is an impossible variable to consider in this survey, as for every WWTP considered, any substantial rainfall does result in the increase of inflow, due to the statewide problem of aged infrastructure and leaking pipes (Dennis Grecci, CT DEEP, personal communication). Rain occurred only on May 3rd and 4th, 2012, during which time the following WWTPs were sampled: Stamford, Fairfield, Greenwich, Mattabassett and New London. PFAA concentrations measured may reflect input from surface runoff, in which case, the measurements obtained will be a good proxy for the influence of WWTP inflow to the LIS from New York, which is dominated by CSO systems.

LIS effluent loading estimates were obtained from the discharge monitoring report pollutant loading tool (US EPA) with data obtained from the US National Estuaries Program, available online at <https://cfpub.epa.gov/dmr/>. The total average daily flow from all treated effluent ranged from $2.98 \times 10^9 \text{ L day}^{-1}$ to $3.92 \times 10^9 \text{ L day}^{-1}$ between 2011 and 2014; an average daily input of 3.3×10^9 . Based on an average 3.3 trillion liters of treated wastewater entering the LIS watershed daily, and the minimum and maximum concentrations measured, the annual total PFAA (sum of all PFAA congeners detected) mass flow into the LIS from WWTPs was estimated at 140 – 1,460 g/day or 50 - 530 kg/year (Figure 3.4). The mass flows estimated in this study are lower, though in a similar order of magnitude, in comparison to that estimated for the heavily urban impacted River Seine, where Labadie *et al.* estimated a mass flow of 485 kg/year (39). However, the annual PFOA mass flow in the same study for the River Seine was estimated at 14 kg/year, whereas the mass flow into the LIS from WWTP effluent was estimated at an average of 43 kg/year (7 – 77 kg/year). Both this study and the River Seine study found considerably lower mass flows than those in the River Rhine (approximately 17 tons/year) (61).

The predominance of shorter chained PFCAs and PFSA ($\leq C_8$) in the effluent emitted to the LIS will predictably result in a mass flow that is conserved due the higher solubilities, as well as the environmentally stable chemical nature of these compounds. This is a reasonable assumption given that PFAA mass flows have been shown to be conserved in the Swiss Glatt River valley (46). The LIS has been described as a partially- to well-mixed estuary (62). Assuming complete mixing of the estimated Σ PFAA annual mass loading in the LIS, and no significant loss to sinking particulates and burial

in sediments, the approximated Σ PFAA (sum of PFAA congeners) concentration flowing out from the LIS towards the Rhode Island Sound and/or New England shelf waters would average at 3 ng/L (1.4-5.4 ng/L range based on lowest/highest observed concentrations and a steady state LIS volume of 18 trillion gallons). Additionally, the PFOA/PFOS ratios range from 3:1 in the spring and 1:1 in the summer. These values and observations are remarkably consistent with the Σ PFAA values and PFOA:PFOS ratios reported by Benskin *et al.* (2012) (63). PFCAs and PFSAAs were measured in surface waters in the Northeast Atlantic during a 2009 Endeavor Cruise (Namibia to Narragansett, RI), where Σ PFAAs were measured at approximately 1 ng/L at station 34, located in the Rhode Island Sound. Furthermore, the PFOA:PFOS ratio at this station was reported was approximately 2:1, indicative of samples in the Northern hemisphere, compared with ~ 1 around the equator, and ≤ 1 in the southern hemisphere (63). It should be noted that the Endeavor cruise was conducted in July 2009; therefore a major assumption made with the comparison to the data in this study and the data of Benskin *et al.*, is an overall annual regularity in the concentration and volume of PFAA influx to the LIS.

Finally, Benskin *et al.* noted that Σ PFAA concentrations (5.8 ng/L) detected in Narragansett Bay (NB), with higher contributions from PFOS, (PFOA:PFOS $\ll 1$) may reflect inflow from the Long Island Sound. However our data do not support this hypothesis, and suspect that the higher PFAAs detected at the NB station are due to sources more local to that station.

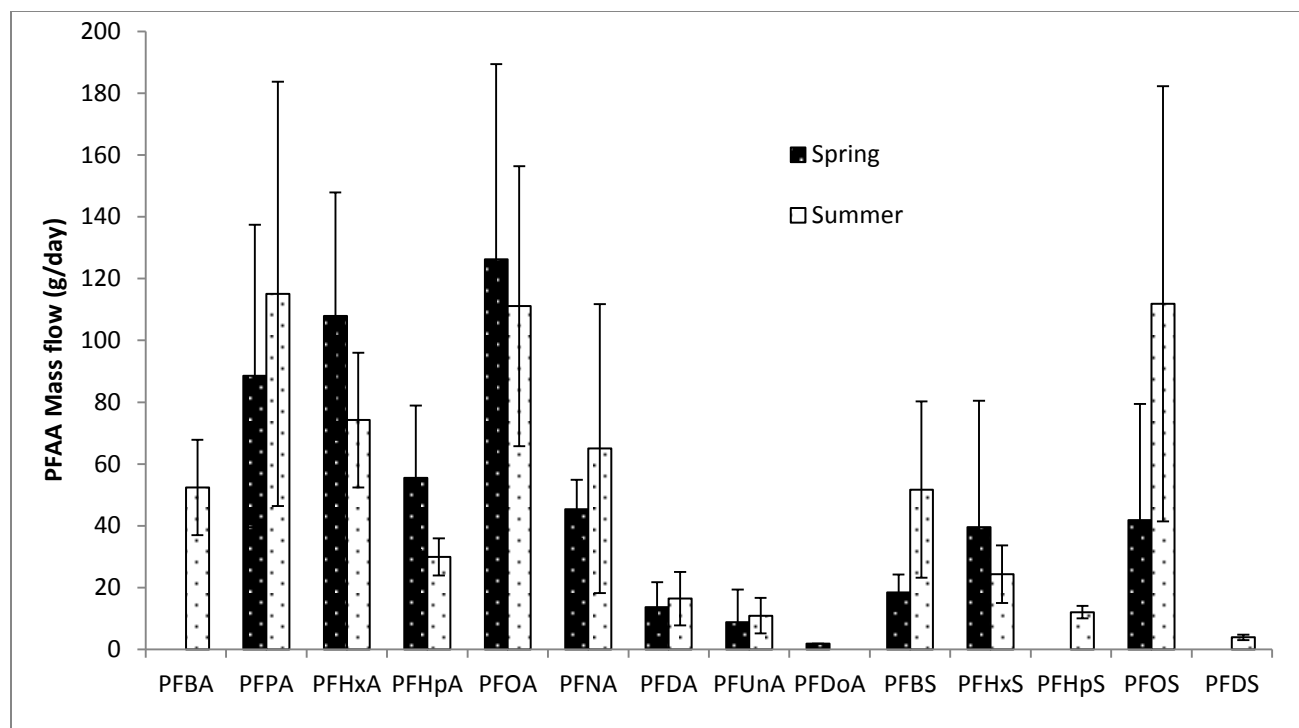


Figure 3.4: Estimated concentrations (average \pm range) of total PFAA loadings to the LIS (g/day).

3.3.3 PFAAs associated with the particulate fraction

The suspended particulates present in the final effluent were separately collected in this study, and were extracted and analyzed in order to determine 1) the PFAA compounds that are associated with the SPM fraction, and 2) investigate partitioning behaviors. The average concentrations associated with each fraction (dissolved vs. efSPM) in the final effluent samples are depicted in Figure 3.5. Individual concentrations of PFAAs associated with the efSPM fraction are reported in Appendix Tables A6 and A7. It should be noted here that the terms ‘dissolved’ and ‘particulate’ are operationally defined as those which pass through or are retained on a filter with nominal pore size of 0.45 μm . As would be expected due to increased hydrophobicity and thus decreasing

solubility, only the longer chained ($\geq C_6$) PFAAs were detected in the particulate fraction, with the longest chained PFAAs observed, PFUnA and PFDoA (with 10 and 11 fully fluorinated carbon chains), found only associated with the particulate phase.

The predominant PFAA in the efSPM fraction was PFOS, seen in 100% of summer and 45% of spring samples and in the highest concentrations. This result is consistent with the literature as PFOS has been reported as the predominant analyte in sewage sludge samples (34, 52, 59). Overall there was greater detection of PFAAs in the summer compared to the spring, with increased detection of PFNA, PFDA and PFOS. Conversely, the % detection of PFOA, PFUnA and PFHxS decreased in the summer. PFUnA was detected more frequently in the spring, and PFDoA was only detected once, also in the spring.

Data from the elemental analysis of the efSPM collected from the 5 WWTPs that were surveyed in spring and summer show, on average, an increase in the concentration of particulate carbon and nitrogen (Table 3.4). In addition, overall averages of measured efSPM concentrations also increase in the summer (8.4 mg/L (2.4-18.5 mg/L) compared to 3.8 mg/L (0.6-7.6 mg/L), and WWTP daily flow rate decreased (Tables 3.1 and 3.2), leading to longer hydraulic detention times, factors which could explain the increased detection of efSPM associated PFNA, PFDA and PFOS in the summer survey. Contrary to the other WWTPs surveyed in both seasons, Plant A did show a decrease in summer efSPM concentration as well as a decrease in particulate carbon and nitrogen, however, it should be noted that the longest chained PFCAs detected (PFUnA and PFDoA) were both detected at plant A during the spring survey, consistent with increased long chained PFAA presence in final effluent

correlating with efSPM concentration. With a substantial proportion of PFAAs detected in the particulate fraction, approximately 50% of PFDA, PFUnA and PFOS, as well as 100% for PFDoA and PFDS, it is clear that filtering effluent samples for extraction and analysis, without additionally considering the proportion of PFAAs retained on the filter, can substantially underestimate the concentrations of PFAAs present.

3.3.3.1 Filtration artifact

The potential for longer chained PFAAs to adhere to the filter, due to their increasing hydrophobicity, was investigated in order to ascertain any bias to the data caused by filtration artifacts. Concerns regarding potential for filter artifact were investigated by Labadie *et al.* (39) using glass fiber (GF/F) and nylon filters with PFAAs spiked in ultrapure water at 10 ng/L. Results of this study indicated that GF/F filters performed better than nylon, showing a lesser retention of PFAAs, yet longer chained PFAAs, particularly C₁₁-C₁₄ PFCAs and PFDS showed sorption in the 10-30% range. In this study, PP filters with a nominal pore size of 0.45 µm were utilized due to the reported potential of PFAA target loss via sorption to glass, which result in PP sample collection containers being favored over glass (45). Retention of PFAAs on the PP filters used in this study was determined according to the procedure reported by Labadie and Chevreuil (39) by filtering ultrapure water spiked with PFAAs, however in this study the PFAAs were spiked at a higher concentration (30 ng/L compared to 10 ng/L) to better reflect the levels of PFAAs observed in the field samples.

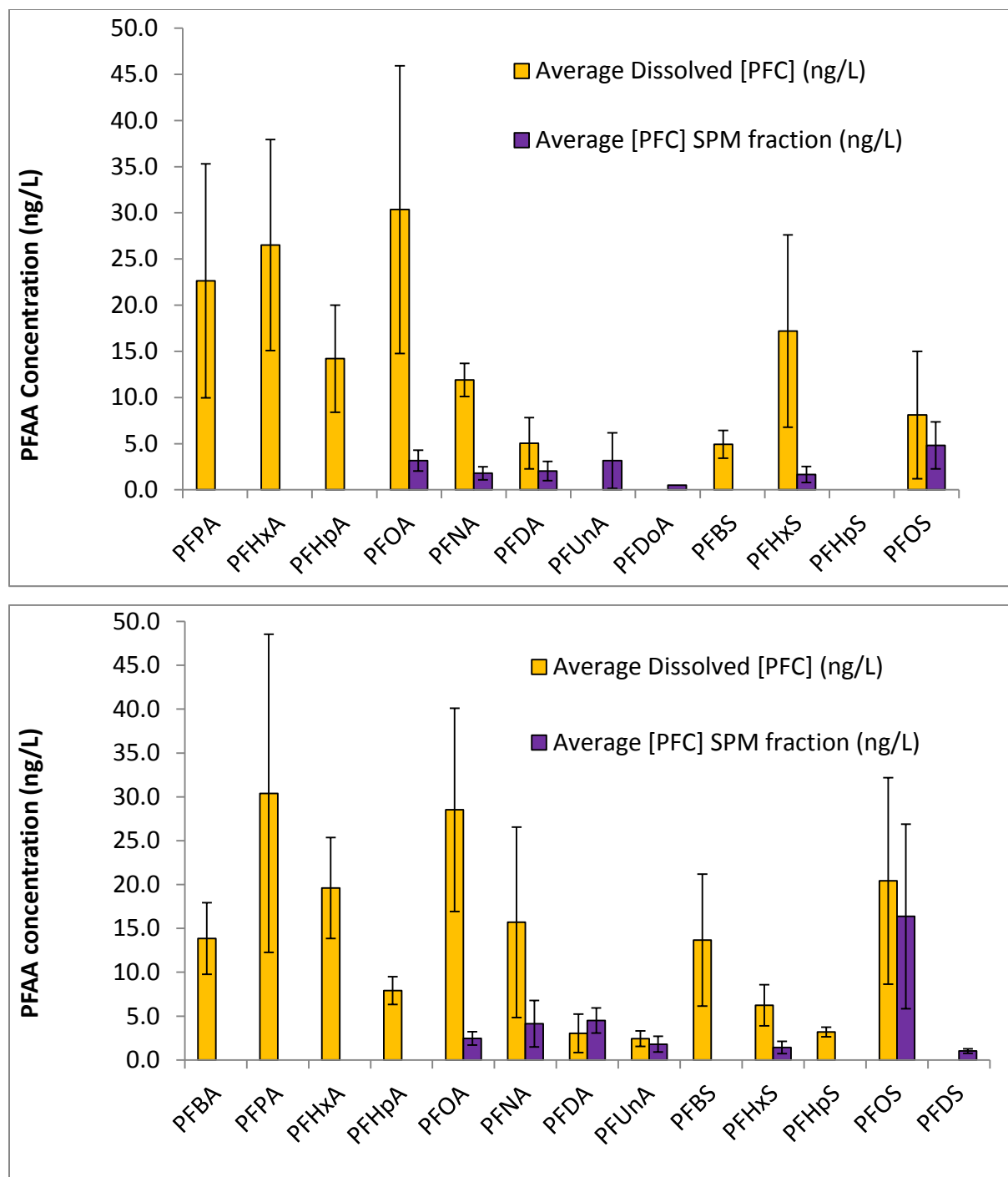


Figure 3.5: Spring (top) and summer (bottom) average effluent PFAA concentrations distributed between the dissolved and suspended particulate phases. Error bars are standard deviation. For PFAAs associated with SPM, concentrations also given in ng/L.

Table 3.4: Elemental analysis of efSPM collected from 5 WWTPs during both spring and summer sampling surveys; total carbon and nitrogen concentrations ($\mu\text{g/mL}$).

WWTP	Spring :	Summer:
	efSPM Carbon efSPM Nitrogen	efSPM Carbon efSPM-Nitrogen
A	3.19	2.04
	0.47	0.32
C	1.39	1.7
	0.24	0.32
G	1.66	3.35
	0.31	0.58
I	2.58	3.37
	0.43	0.62
K	3.12	3.62
	0.48	0.61

No contamination was observed from the PP filters due to thorough MeOH rinsing prior to use. Retention of PFAAs on the filter was found for longer chained PFCAs and PFSAAs ($\geq C_8$), with % retention increasing with chain length; 1.4% and 2.0% of PFOA and PFNA respectively, and 11% of PFDA and PFOS was found to be retained by the filter, and much greater proportions (20 - 35% from PFCAs C_{10} - C_{13} , and 45% of PFDS) retained by the filter of PFAAs possessing 10 or more fully fluorinated carbons, additionally with much greater standard deviations (Figure 3.6). These results are consistent with the report from Labadie and Chevreuil (2011b) (39), which are also shown (Figure 3.6) for comparison. In their study, Labadie and Chevreuil concluded that GF/F filters performed better than nylon, with a reduced PFAA retention, which was confirmed by Chandramouli *et al.* (2015) (64). The PP filters in this study perform as well as the GF/F filters for the PFCAs, however, the adherence of PFDS to the PP filters was similar to that reported for the nylon filters (>40%), far greater than reported for the GF/F filters (24%). Although the nominal pore size of the nylon filters was not given, the

nominal pore size of the GF/F filters is 0.7 μm , thus pore size may account contribute to better performance seen by the GF/F for PFDS.

The average % PFAA retention by PP filters was determined to increase in a linear fashion for PFCAs $\text{C}_8\text{-C}_{11}$, as well as approximately for PFSA C_6 , C_8 and C_{10} , with the % retention for PFSA estimated to be great than the PFCA counterpart, with a consistently proportional increase with increasing perfluoroalkyl chain length (Figure 3.7). These observations are in agreement with reports that PFAAs partition to particulates with a constant log-unit increase, ranging from 0.5-0.8 per fluoro-alkyl moiety (35, 36, 38, 65), with PFSA having higher partitioning coefficients compared to their PFCA counterpart with the equal number of fluorinated carbons (36, 38).

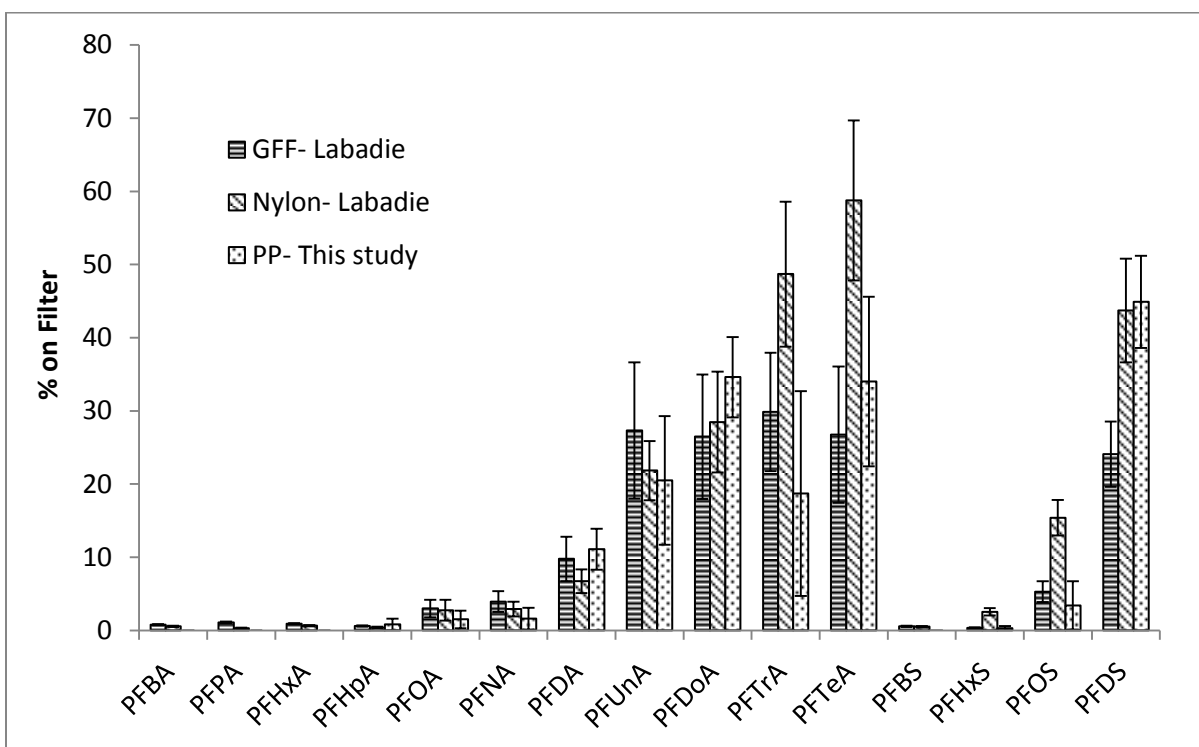


Figure 3.6: Retention of PFAAs on polypropylene (PP) filters (this study, $n=4$) compared with previously published data comparing PFAA retention on glass fiber (GFF) and nylon filters ($n=3$) (39). Results given are mean \pm standard deviation.

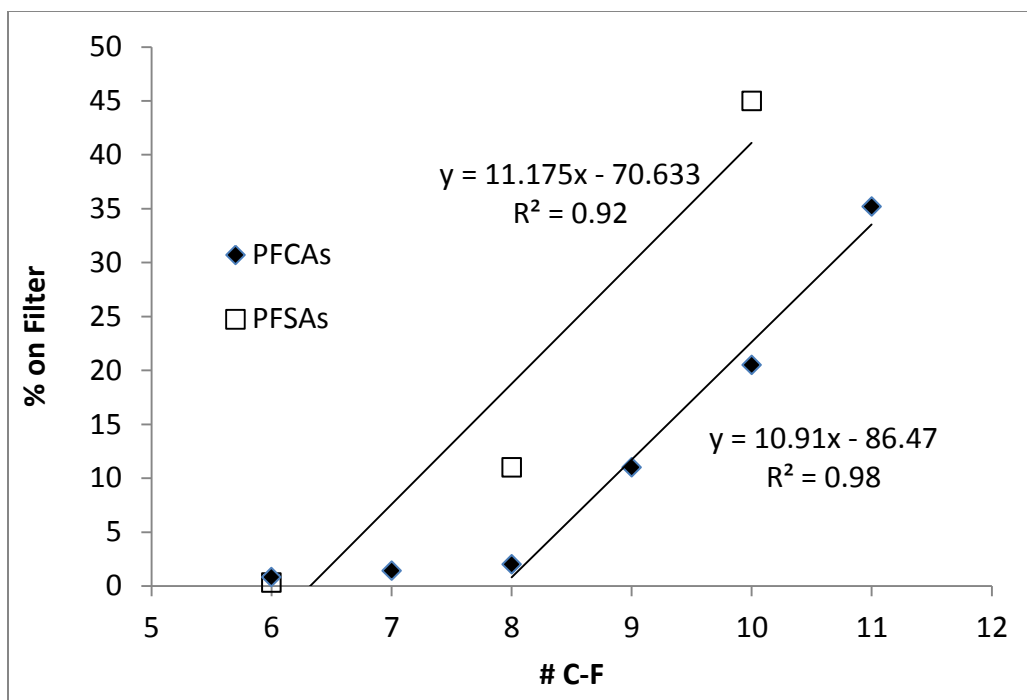


Figure 3.7: Average % retention of PFAAs on polypropylene (PP) filters as a function of perfluoroalkyl chain length for PFSA_s C_{6, 8, 10} (□) and PFC_s C₄₋₁₄ (◆) in this study.

Filtration artifacts could lead to an overestimation of particle-bound PFCs and an underestimation of dissolved phase PFAAs; PFAA concentrations reported in this study therefore have been the sum of dissolved and particulate bound PFAAs. In order to ascertain the proportion of particulate bound (efSPM bound) PFAAs, the amount of dissolved PFAA that would be retained on the filter was calculated from the measured dissolved and particulate levels, and the expected filtration artifact determined by the spiking experiments.

Investigations by Chandramouli *et al.* (64) found filter artifacts for PFAAs to be consistent for different concentration levels, as well as for various matrix types, including ultrapure and effluent waters. The filtration artifacts determined in this study

therefore can be confidently applied to correct for the amount of PFAA in the dissolved and particulate phases, by knowing the measured level of the target analyte in the dissolved phase and applying the filtration artifact.

3.3.3.2 Estimations of particle-water distribution coefficient

In effluent samples where target PFAAs were detected in both dissolved and efSPM phases, experimental partitioning constants (K 's) were derived, following correction for filtration artifacts, using the following equations;

$$K_d = \frac{C_{efSPM}}{C_{diss}}$$

Where C_{efSPM} is the concentration PFAA detected in the efSPM phase, corrected for filter artifact (ng/Kg), and C_{diss} is the concentration in the dissolved phase (ng/L). K_{oc} was calculated using the measured organic carbon concentrations to derive the efSPM fraction of organic carbon (f_{oc});

$$K_{oc} = K_d \times \frac{100}{F_{oc}}$$

Log K_d and K_{oc} values calculated were determined to increase in a linear fashion with increasing fluoro-carbon chain length, agreeing well with many other field-based reports (35, 36, 38) as well as lab-based partitioning studies (65), and which reflect the importance of the hydrophobic interaction in the sorption process (66). Linear regressions of both PFCAs and PFSAAs determined the log unit difference for each additional $-CF_2-$ moiety as 0.42 and 0.33 respectively, which is similar to but less than

the 0.5-0.8 range reported in these studies (Figure 3.8). PFSA were on average observed to partitioning more strongly to efSPM compared to the PFCA with the same number of perfluorinated carbons, with log unit difference of 0.4; however this difference was not statistically significant. A similar log unit difference was derived by Ahrens *et al.* (38) for SPM in Tokyo Bay, where a difference of 0.85 was reported, and smaller log unit difference of 0.23 log units was determined experimentally by Higgins and Luthy (65). Labadie and Chevreuil (36) reported a log unit difference between PFSA and PFCA of 0.78, however it should be noted that in their report, the linear regression performed used the number of carbons in perfluoroalkyl chain and not the number of fluorinated carbons; for the PFCA, one of the carbons in the chain belongs to the carboxylic head group, therefore the number of fluorinated carbons is one less than the carbon chain length for which the PFCA is named (e.g. 7 -CF₂- moieties for PFOS). Reanalysis of the data from Labadie and Chevreuil (36) using the number of fluorinated carbons in each analyte found no difference in the partitioning extents of PFCA and PFSA, which was also reported for a statistical re-analysis of Higgins and Luthy's data by Rayne and Forest (67).

Higgins and Luthy (65) found that PFAA sorption onto sediment was strongly correlated with the sediment organic carbon fraction (f_{oc}). Log K_{oc} values derived in this study from 4.62 – 5.76 for PFCA (PFOA-PFUnA), and 4.55 - 5.21 from PFSA (PFHxS and PFOS) (Table 3.5). While the log-unit differences observed, per fluoroalkyl moiety for PFCA and PFSA, and between the carboxylate and sulfonate functional group compounds, the values of the partitioning constants are 2 orders of magnitude greater those derived by Higgins and Luthy (65) of 2.06 - 3.30 (PFOA-PFUnA) and 2.57 (PFOS)

investigating partitioning behaviors using natural sediment samples. Other studies have shown higher partitioning constants derived for SPM samples compared with bed sediments in field based studies; Ahrens *et al.* (38) derived $\log K_{oc}$ values ranging from 3.5 - 5.1 (PFOA-PFUnA), and 3.7 - 4.8 for PFHxS-PFOS) for SPM samples in Tokyo Bay, approximately 1 order of magnitude greater than the $\log K_{oc}$ values derived for sediment samples in the same region. However, our field based $\log K_{oc}$ values are on average 1 order of magnitude greater than those reported by Ahrens *et al.* (38) even following correction for any potential filter artifact. A brief summary of reported $\log K_d$'s and K_{oc} 's is given for comparison (Table 3.6).

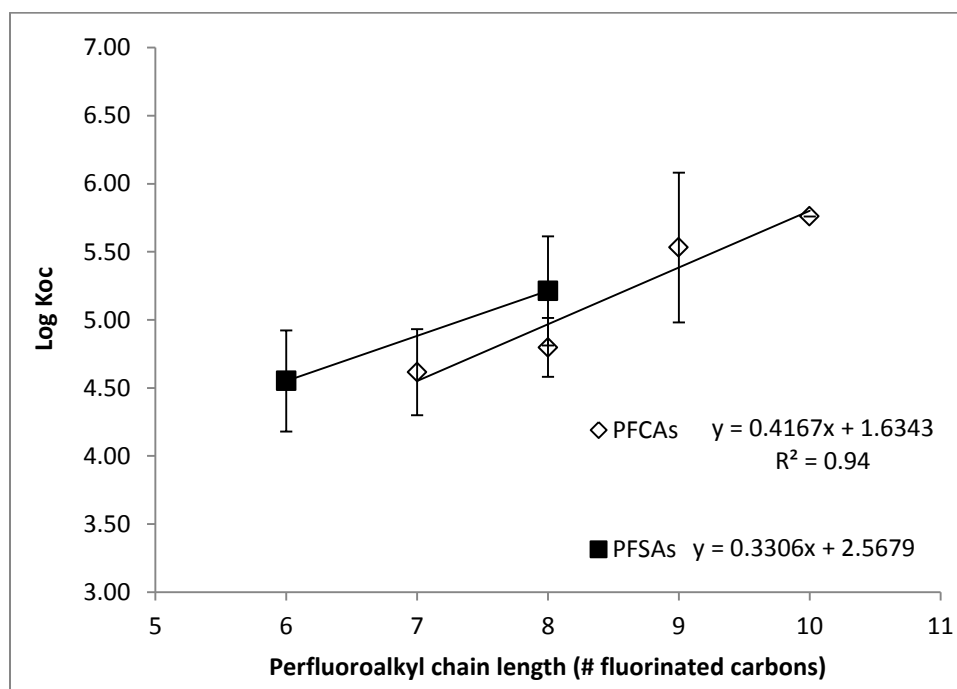


Figure 3.8: Effluent suspended particulate organic carbon-water distribution coefficients derived for PFCAs (■) and PFSA (◇) with fully fluorinated carbon chain lengths of C_{7-11} and $C_{6,8}$ respectively. Values are the mean, error bars are standard deviations. There are no error bars for PFUnA due to $n=1$ for this analyte.

Table 3.5: Average partitioning constant values (standard deviation) derived from data obtained from summer alone (top) and for spring and summer (bottom). K_d =particle-water distribution; K_{oc} =particulate organic carbon-water distribution. All distribution coefficients have units of L/Kg.

PFC	#C-F	Log K_d	Log K_{oc}	n
PFOA	7	4.19 (0.24)	4.59 (0.21)	4
PFNA	8	4.22 (0.37)	4.88 (0.23)	5
PFDA	9	5.32 (0.50)	5.79 (0.48)	2
PFUnA	10	5.24	5.76	1
PFHxS	6	4.35 (0.07)	4.89 (0.09)	2
PFOS	8	4.61 (0.56)	5.24 (0.46)	11
PFC	#C-F	Log K_d	Log K_{oc}	n
PFOA	7	Nd	4.62 (0.32)	9
PFNA	8	Nd	4.80 (0.22)	7
PFDA	9	Nd	5.53 (0.55)	3
PFUnA	10	Nd	5.76	1
PFHxS	6	Nd	4.55 (0.37)	5
PFOS	8	Nd	5.21 (0.40)	15

Nd = Not determined for spring samples.

Table 3.6:Comparison of field derived Log K_{OC} values (\pm standard deviation).

Sample region Solid phase	PFC	Log K _D	Log K _{OC}	n	Reference
WWTPs, Greece. Wastewater effluent- primary sludge (PFCAs) Mixed liquor (PFSAs)	PFOA PFNA PFDA PFUnA PFHxS PFOS	3.09 3.36 4.11 3.89 3.63 3.73	Not reported	5	(34)
WWTPs Tianjin, China. Wastewater-sludge	PFBA PFPA PFHxA PFHpA PFOA PFNA PFDA PFUnA PFDoA PFHxS PFOS	0.62 1.5 2.0 2.28 2.48 2.69 3.05 3.55 3.87 3.09 3.29	Not reported	12	(26)
River Seine, France. River SPM	PFHxS PFOS	2.1 \pm 0.4 3.1 \pm 0.3	3.2 \pm 0.6 4.0 \pm 0.6	16	(39)
South Korea. River SPM	PFOA PFOS	0.21 \pm 0.4 0.53 \pm 0.4	1.6 \pm 0.6 1.9 \pm 0.7	17	(68)
Tokyo Bay, Japan. Estuary SPM	PFHpA PFOA PFNA PFDA PFUnA PFHxS PFOS	1.9 \pm 0.002 2.4 \pm 0.1 2.9 \pm 0.1 3.5 \pm 0.2 4.2 \pm 0.2 2.6 \pm 0.4 3.7 \pm 0.1	2.9 \pm 0.002 3.5 \pm 0.1 4.0 \pm 0.1 4.6 \pm 0.1 5.1 \pm 0.1 3.7 \pm 0.3 4.8 \pm 0.1	6	(38)
Tokyo Bay, Japan. Estuary bed sediment	PFOA PFNA PFDA PFUnA PFHxS PFOS	0.04 \pm 0.03 0.6 \pm 0.1 1.8 \pm 0.1 3.0 \pm 0.1 1.8 \pm 0.1 2.1 \pm 0.1	1.9 \pm 0.1 2.4 \pm 0.1 3.6 \pm 0.1 4.8 \pm 0.2 3.1 \pm 0.1 3.8 \pm 0.1	6	(38)
Orge River, France. Bed sediment	PFHxA PFHpA PFNA PFDA PFUnA PFDoA PFHxS PFHpS PFOS	0.8 \pm 0.0 0.8 \pm 0.1 1.5 \pm 0.1 2.4 \pm 0.2 3.4 \pm 0.1 4.3 \pm 0.2 0.9 \pm 0.0 1.6 \pm 0.1 2.4 \pm 0.2	2.1 \pm 0.2 2.1 \pm 0.2 2.9 \pm 0.1 3.8 \pm 0.2 4.7 \pm 0.1 5.6 \pm 0.2 2.2 \pm 0.1 2.9 \pm 0.1 3.7 \pm 0.2	3	(36)
Rivers, lakes canals, The Netherlands. River bed sediment	PFOS PFOA PFNA	2.46 \pm 0.33 1.83 \pm 0.40 2.89 \pm 0.53	3.16 \pm 0.28 2.63 \pm 0.34 3.69 \pm 0.52	19	(35)
Liao River, China River bed sediment Taihu Lake, China. Lake bed sediment	PFOA PFHxS PFOA PFOS	Not reported 2.16 \pm 0.54 2.28 \pm 0.55 2.88 \pm 0.62	2.28 \pm 0.62 2.16 \pm 0.54 2.28 \pm 0.55 2.88 \pm 0.62	NR	(69)

Under-sampling could account for the relatively high partitioning constants derived in this study these results, due to the low concentration of efSPM, ranging from 2.4 - 18.5 mg/L. Laboratory based partitioning experiments, and field based sediment derived partitioning constants, utilize solid samples with masses in the range of 1–5 grams, therefore the higher SPM derived K_d values may be due to the low concentration of the solid phase, given that the units of the partitioning constants are given in L of water per Kg solid. Similar low SPM concentrations (3.2 - 5.0 mg/L) were measured in Tokyo Bay by Ahrens *et al.* (38) in conjunction with higher log K_{oc} 's for PFAA water-SPM partitioning.

Another potential explanation for the relatively high partitioning constants derived in this study is methodological. PFAA concentrations were determined by filtering using 0.45 μm nominal pore size PP filters. Samples obtained from elemental analysis utilized GF/F filters, which have a nominal pore size of 0.7 μm . As discussed previously in reference to the filter artifact, the pore size may have had an effect on the increased retention of longer chained PFAAs, as seen with PFDS. Although PFAA concentrations had been accounted from with the artifact correction, the GF/F concentration of efSPM carbon or organic carbon may have been underestimated relative to the amount captured by the PP filters, leading to an artificially inflated efSPM associated PFAA concentration. However, experiments performed on both PP and GF/F filters confirmed that there was no statistically significant difference in the SPM mass retained by both filter types.

With pK_a values lower than 3 for PFCA and even lower for PFSA, PFAAs exist predominantly in the anion state at the pH levels found in WWTPs (34). PFCA and

PFSA partitioning has been reported to increase with the presence of divalent ions such as Ca^{2+} , and with decreasing pH (65, 66). A review on the general composition of grey wastewater (wastewater without any inputs from toilets) reported Ca^{2+} concentration ranges from 3.5 - 7.9 mg/L, Mg^{2+} (1.4 - 2.3 mg/L) and monovalent ions including Na^+ (7.4 – 18 g/L) (70). The presence of divalent ions such as Ca^{2+} facilitates sorption of PFAAs to organic carbon by reducing the overall negative charge on the organic matter surface and reducing the repulsion from the anionic head group thereby acting as a cation bridge (65).

For each WWTP investigated, the flow rates and hydraulic retention times of the secondary clarifiers was such that effluent remained at the secondary clarifying stage for a minimum of 4 hours. Sorption studies with wastewater sludge determined sorption equilibrium to be achieved after approximately 4 hours (71). Ahrens *et al.* (38) suggested that the higher K_d 's obtained for SPM in Tokyo Bay may be due to disequilibrium, however that is not likely to be a factor in this study given that most samples were 24 hour composites. Arvaniti *et al.* (34) found the partitioning of PFAAs to WWTP sludge samples found greater K_d 's for primary sludge compared to mixed liquor or secondary sludge for PFCAs $\text{C}_8\text{-C}_{11}$, ranging from 3.09 - 4.11, but higher K_d 's for PFSA's in mixed liquor; 3.63 and 3.73 for PFHxS and PFOS respectively, strongly suggesting that the type of sludge affects the degree of PFC sorption.

PFAAs have been shown to preferentially sorb to proteinaceous rather than lipidic environments (67). Wastewater effluent particulate matter is a mixture of organic detritus and microorganisms such as bacteria and algae (41). Elemental analysis of the efSPM samples from the 12 CT WWTPs found C/N ratios to be 6 - 8. Proteins are the

primary nitrogen compounds of phytoplankton and zooplankton, and have C/N ratios ranging between 4 and 10 (72). Thus a plausible interpretation of the data obtained in this study is that the suspended particulate matter found in discharging stream of the final effluent, has a higher protein composition compared to riverine SPM or bed sediments, due to the SPM consisting primarily of microorganisms, or increased levels of microbial exudates following secondary treatment, leading to higher PFAA sorption. This could also account for the fact that there were a greater number of PFAAs detected in the efSPM phase in the summer months, as the higher ($\sim 10^{\circ}\text{C}$ difference) temperatures in the WWTP water during the secondary clarifying stage will likely promote greater rates of microorganism growth. Though the majority of samples collected were 24 hour flow-proportional, the C/N signatures were the same in the 4 grab samples obtained, suggesting a consistent composition of effluent particulate matter. PFAAs have been shown to preferentially concentrate in blood and liver, and in vitro studies have reported that they associate strongly with proteins including serum albumin. Interaction of PFAAs with proteins within an organism has been proposed as the mechanism of PFAA bioaccumulation, due to the similarity between the PFAA molecular structures to endogenous fatty acids (81).

The partition coefficients derived in this study, describing the tendency of longer chained PFAAs to sorb to efSPM, are the first partitioning parameters derived for final effluent suspended particulates. While the studies of Ahrens *et al.* and Labadie and Chevreuil both reported higher magnitude partitioning coefficients for SPM in riverine and estuarine environments, this is the first study that has investigated efSPM partitioning, and so it is not possible to directly compare the data obtained in this study

with other literature values. Only one other study has specifically investigated efSPM-water partitioning in field samples; Vierke *et al.* (40) investigated particle-water PFAA distributions *in situ*, within an aeration basin and within primary and secondary clarifying units. The particle-water partitioning coefficients derived were lower in magnitude, with log K_d values reported of 2.11 and 2.34 for PFOA and PFOS respectively in the aeration basin. However, the Vierke *et al.* also reported that the organic carbon content of the effluent particulates in their study to be 0.27% in the aeration tank, therefore the particulate matter investigated in their study is fundamentally different in chemical nature to the particulate matter in the final effluent samples in this study, in which the organic carbon content was an average of 30%. The data for this study indicates that the final effluent SPM matter is composed mainly of microorganisms. The small sizes of the efSPM together with the high protein content are the predominant factors contributing to the larger magnitude partitioning coefficients derived.

3.4 Conclusions

Perfluorinated alkyl compounds were detected in the final effluent samples obtained from a survey of 12 CT shoreline WWTPs in spring and summer 2012. The dominant PFCA and PFSA were PFOA and PFOS, although shorter chained PFCAs (PFPA and PFHxA) were also found to be present in similar and sometime in greater concentration than PFOS, which could be reflective of the EPA's industrial stewardship program restricting the use and manufacture of longer (>C₈) chained PFAAs. The WWTP with the overall greatest PFAA mass flow to the LIS (due to both higher daily flow rate and \sum PFAA concentrations) was Stamford, located in the most western part of the LIS. This raises to important questions for further research: 1) What is the potential

for PFAA input from other CT WWTPs not included in this survey, located within much greater population densities and with much higher daily flow rates, situated in the central and northern parts of the state, and which discharge into the major tributaries thus ultimately impact the LIS. 2) Due to reduced tidal flushing of the western basin, numerous water quality concerns affect the WLIS, including higher nutrient loadings and seasonal hypoxic event. With the potential for PFAA loading to be greater in this more heavily populated region, questions may be raised regarding what the potential impact of PFAAs to the ecosystems in the WLIS may be, and whether these effects be exacerbated by seasonal water quality issues.

The overall average annual total PFAA mass loading to the LIS, from an assumed input of 3.3 trillion liters/day WWTP effluent, is estimated at 50 - 530 Kg/year. Assuming complete mixing in the LIS, the average water concentration in the Sound was calculated to be 3 ng/L; however, it is likely that concentrations are much greater closer to the shorelines. These values reported are much lower than the predicted no-effect concentration guideline value for protecting marine wildlife of 2.5 µg/L (73) however since longer chained PFAAs are known to bioaccumulate and biomagnify, future research concerning the concentration of PFAAs in biota important to the food chain in the LIS is warranted, particularly for ecosystems located closer to the shoreline, to effluent discharge points, and in locations with reduced tidal flushing. As mass is highly likely to be conserved, the estimated annual discharge of PFAAs from the LIS to open ocean waters, based on an estimated water transport mean flow at the race (Eastern LIS) of 525m³/s (62), is on the average order of 20 – 60 Kg/year.

Longer chained PFAAs ($\geq C_8$ PFCAs and $\geq C_6$ PFSAAs) were found to be associated with the suspected particulates present in the wastewater effluent samples, due to the preference of the increasingly hydrophobic fluorocarbon chain to partition to organic matter, in particular organic carbon, rather than being in the 'freely dissolved' fraction (67). PFOS in particular was found to have close to 50% of total mass associated with the efSPM, and longer chained PFCAs and PFSAs were detected only in the particulate phase. It can be concluded that sample filtration prior to extraction without the additional extraction and analysis of the filter will lead to a significant underestimation of the Σ PFAAs present in a sample.

Partitioning was determined to be a function of chain length, with a log unit increase in log K_{oc} values of 0.4 per fluoroalkyl moiety, consistent with literature reports, however the partitioning values derived were found to be 1 - 2 orders of magnitude greater than previously published values, however the partitioning data presented in this study is the first for PFAAs associated with effluent derived suspended particulate matter from composite samples of final effluent, therefore no direct comparison exists. The low SPM concentrations in this study, compared to partitioning studies performed with bed sediment samples or with sewage sludge samples, may be a factor in the calculation of higher partitioning constants; however, larger K_d 's derived were also for SPM in other studies (38, 39). Particulate size will likely influence the adsorption capacities of the SPM for PFAAs, as suspended particulates of smaller size will have increased surface area. Larger K_d 's derived for the SPM studies may also be a function of the inverse dependency of K_d on particle concentration (known as the particle concentration effect) (74).

Elemental analysis of the efSPM determined a consistent C/N ratio of 6 - 8, consistent with the high protein signature indicating that WWTP microorganisms make up a large proportion of the efSPM, which may be significantly increasing the adsorption capacities of efSPM to PFAAs due to the proteophilic nature of these compounds. Further research is necessary to further elucidate the relationship between particulate nature and PFAA partitioning. The sorption of longer chained, more bioaccumulative PFAAs such as PFOS to efSPM is of particular concern, as efSPM has been reported to be preferentially assimilated by benthic biota (41). This may represent an important vector in the transport of these compounds to higher trophic levels. Utilizing stable isotopes as tracers of sewage derived particulate organic matter, Ramirez-Alvarez *et al.* determined that effluent particulate matter supplied a significant portion of organic carbon assimilated by benthic macroinvertebrate species in areas as far away at 26 Km from sewage input in the Southern California Bight (75). Effluent derived SPM is a high quality food source, and in this regard the traditional assumption that particle-bound pollutants are not considered to be bioavailable, must be reassessed. In fact, PFAAs associated with the sediment have already been reported to be bioavailable (76) and a major source of PFAAs to an aquatic food web (77). Of particular concern in this region is biota of commercial importance such as oysters, since these bivalves are capable of filtering very high volumes of water and SPM per day. Future research on the role of efSPM as a vector of PFAAS to the food chain is merited.

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Occurrence and Distribution of Perfluoroalkyl Acids along the Housatonic River Estuary under Contrasting Hydrological Regimes

Abstract

The presence and partitioning behaviors of perfluoroalkyl acids (PFAAs), C₄ – C₁₄ perfluorocarboxylic acids (PFCAs) and C₄, C₆₋₈ and C₁₀ perfluorosulfonic acids (PFSAs), were investigated in river water, estuarine waters, suspended particulate matter (SPM), bed sediments and oysters of the Housatonic River and estuary, Connecticut. Wastewater treatment plant (WWTP) effluent was shown to be a major source of PFAAs to the Housatonic River, as well as to the Naugatuck River (the major tributary river to the Housatonic). PFOS was the dominant PFAA in the river water samples, followed by PFOA and PFPA, with concentrations ranging from 2.2 - 13.7, 1.3 - 7.5 and 0.7 - 4.6 ng L⁻¹ respectively. Total PFAA concentrations in the river water ranged from 8.7 to 80.4 ng L⁻¹, with higher concentrations detected up river, and lower

concentrations at the river mouth, consistent with mixing of fresh Housatonic River waters with saline waters from the Long Island Sound estuary.

Surface water PFAA concentrations decreased in the spring of 2013, with increased river flow hydrology, suggesting that point sources such as WWTP effluent discharge are the predominant source of PFAAs to the river estuary. Although concentrations were generally lower in river water samples during high volume river discharge, the flow rate of the river was 10 times higher in the spring, so overall Σ PFAA mass flow increased over 3 times under high river flow conditions. Therefore contributions of PFAAs from non-point sources, such as atmospheric deposition and urban run-off, cannot be excluded. Total PFAA mass flux from the Housatonic River to the Long Island Sound was estimated to be an average (range) of 72 (61 – 87) g/day during the summer low flow, and 273 (205 – 342) g/day under the spring seasonal high river discharge hydrology.

PFOS was the only PFAA detected associated with the SPM phase in river water samples during summer low flow conditions; Log K_{oc} values obtained were much higher than partitioning values previously reported and did not vary with salinity. Several PFAAs were determined in the sediments near the river mouth, as well as directly downstream of the effluent discharge points of two WWTPs investigated; PFOS was the predominant PFAA in sediments, similar to the SPM phase. Results indicate that although the major portion of PFAA mass flux in the Housatonic River system is in the soluble phase and mass flux is conserved along the salinity gradient of the river-estuary system, a small proportion of longer chained PFAAs ($> C_6$) partition to and are lost to sediments in the proximity of the WWTP discharge and also in the river-estuary

sediments with higher organic carbon. A pilot study using oysters as biomonitors deployed at the Housatonic River mouth resulted in the detection of longer chained PFAAs ($> C_{10}$) indicating that these more bioaccumulative and toxic PFAA congeners are present in this region.

4.1 Introduction

Perfluoroalkyl acids are persistent contaminants that are distributed and detected worldwide in water (1), sediments (2) and biota (3), and are of particular concern due to their various toxic effects and bioaccumulative properties (4). Measurements of perfluoro-alkyl acids (PFAAs) in wastewater effluents have shown that wastewater treatment plants (WWTPs) are point sources of PFAAs to receiving waters (5, 6, 7, 8, 9, 10, 11, 12). Indirect sources of PFAAs include the atmospheric degradation of volatile precursor compounds and subsequent deposition (13). Non-point sources of perfluorochemicals to aquatic systems have also been established by a number of studies, including land application of wastewater (14) and street run-off in urban environments (15). With PFAA concentrations in WWTP effluent markedly higher in areas with higher population densities (16), urban areas are considered as major sources of PFAAs to the aquatic environment.

Perfluorinated compounds in municipal WWTPs receiving inputs from residential areas are most likely originating from the use of fluorochemical containing products such as household cleaners or from products with stain resistance applications (8, 17). Fluorochemicals are also often detected in higher concentrations in WWTP effluents with greater proportions of industrial inputs (5, 18, 19) and are particularly associated

with industries involved with the direct production, processing and dispersion of fluoropolymers (20) as well as industries that utilize fluorochemicals for their chemical and physical properties, including inertness, chemical and thermal stability, surface active nature and stain resistance, including airports (21, 22), and paper or textile treatments, such as carpet manufacturers (10). The conventional WWTP process has proven to be inefficient at removing PFAAs due to the ionic, highly soluble nature of these compounds (6, 8, 19). In addition, a number of mass balance studies on PFAAs within the wastewater treatment process have determined that concentrations of several PFAAs to be higher in effluent than influent (12, 16), suggesting that PFCAs and PFSAAs are formed during the wastewater treatment processes from the degradation of precursor compounds (5).

The discharge of PFAAs from WWTP to local aquatic systems with subsequent investigation into the concentrations, mass flows and biogeochemical dynamics of PFAAs in the receiving water environments has been investigated in only a very few studies. Ahrens *et al.* (10) investigated the PFAAs in both the effluent of several WWTPs and the receiving waters of the River Elbe (Germany) and estimated a mass flow of 950 g/day of Σ PFAAs, concluding that as PFAA concentrations were 5-10 times higher in WWTP effluents than receiving waters, WWTPs were a major source of PFAAs to the River Elbe. Huset *et al.* (8) also concluded that discharge from seven WWTPs were the major sources of PFAAs to the Glatt River (Switzerland) and contributions from non-point sources were not significant. In addition, the authors also found that mass flows of PFAAs within the 35 km Glatt River were conserved, thus

sorption to soils and sediments was not found to be a significant loss mechanism in this fresh water system.

A number of reports have documented the partitioning of PFAAs between suspended particulate phases in both riverine (23, 24) and estuarine (25, 26) systems. Fewer field studies have reported on the partitioning dynamics of PFAAs between water and suspended particulate matter (SPM), and between water and bed sediments, in both fresh riverine waters as well as in the increasingly saline environment as the same river flows towards the estuary. Estuaries are known sinks for river-borne organic contaminants, including other halogenated hydrocarbon pollutants used as herbicides and pesticides, as well as those from industrial wastes such as polychlorinated biphenyls (PCBs) (27).

Few studies have also investigated the impact of river flow on the concentration of PFAAs. Labadie *et al.* (28) found that PFAA concentrations in the heavily urbanized River Seine (Paris, France) negatively correlated with river flow rate, suggesting the predominance of point sources in this system, though the contribution of non-point sources could not be excluded. In contrast, Zushi *et al.* (29) found PFAA concentrations to remain the same, or for some compounds, to slightly increase in river water under increased river discharge thus concluding the significance of non-point sources to total PFAA load in the Tsurumi River (Japan). While the sources of PFAAs to the LIS watershed have been implied (Chapter 3) the quantitative contribution and relative significance to riverine PFAA mass flow has yet to be determined.

This present study is aimed at investigating the presence and partitioning behavior of PFAAs in both fresh and saline waters of the Housatonic River (HR), the second largest source of fresh water flowing into the LIS estuary, under contrasting hydrological regimes of summer low-flow and spring high flow conditions, in order to estimate the mass flux of PFAAs into the LIS, and to determine the relative significance of WWTP point sources and the potential of non-point source contributions. In addition, to investigate the biogeochemical dynamics of PFAAs along the salinity gradient of the Housatonic River and estuary in order to ascertain the influence of salinity on the water-SPM and water-bed sediment partitioning dynamics of PFAAs under field conditions. Finally, a small pilot study was also conducted utilizing oysters deployed at the river mouth in order to compare concentrations determined in water and sediment with biota, and to estimate bioconcentration factors (BCFs) for this regionally economically valuable species.

4.2 Study sites

The Housatonic River (HR) watershed covers an area of approximately 5,123 km² of western Connecticut (CT), ranging north into Massachusetts (MA), and into New York (NY) state in the west. With headwaters originating in southwestern MA, and flowing south for 150 miles, it is the second largest river bringing fresh water into the Long Island Sound (LIS). The HR begins its journey from two upper branches; the East Branch originates in Muddy Pond, between the towns of Hinsdale and Washington, MA and flows 17 miles to meet the West Branch just south of Pittsfield, MA. The West Branch of the HR originates in Pontoosuc Lake, north of Pittsfield. There are eight major tributaries feeding into the main HR; the Williams and Konkapot Rivers which both

originate in MA, the Green River, which originates in NY, and the Ten Mile, Still, Shepaug, Pomperaug and Naugatuck Rivers, which all originate in CT.

The Housatonic watershed consists of 60% forest, 25% urban area, 7% water and 8% agriculture, with the upper part of the watershed being forested with minimal agriculture and the lower region closer to the CT shoreline becoming increasingly residential (30). Historic industrial discharges to the upper Housatonic River and tributaries, in cities including Pittsfield, Danbury and Waterbury, had released pollutants such as PCBs and heavy metals (31) leading to large-scale clean-up and restoration efforts since the 1980's by the EPA (32).

The lower Housatonic River is impacted by several dams; the southernmost dam, located in the north of the towns of Derby and Shelton, truncates the tidal portion of the river (31). The lower Housatonic River, below the Derby-Shelton Dam, is the field region in this study. The HR between the towns of Derby (east bank) and Shelton (west bank) is the sampling location farthest upstream (HR 9), at 20.9 km from the mouth of the river. The river mouth is itself located between the city of Milford on the east and the town of Stratford on the west, and is the southernmost river sampling point (0 km). The river sampling stations were numbered according to their distance along the axis from the river mouth, with station 1 located at the mouth, station 2 at 2.2 km, station 3 at 4 km, station 4 at 6.2 km, station 5 at 8.1 km, and station 8 at 17.1 km upstream (Figure 4.1). Stations 6 and 7 were not sampled due to time constraints. Station 9, located in Derby, was at the confluence of two rivers; the Housatonic was sampled at station 9A, 1.8 km downstream of the Derby dam, and 300 m downstream of the Derby WWTP effluent discharge point. The Naugatuck was sampled 5 m north of the point of

confluence (station 9B) in order to assess the input of PFAAs from the Naugatuck River. Both stations 8 and 9 are the fresh water tidal portions of the HR, with a mean tidal amplitude of 1.5 m.

Six municipal waste water treatment plants (WWTPs) are located along this portion of the lower HR. The Derby WWTP discharges in the northern most point of the study area. The Shelton WWTP is situated directly opposite the Naugatuck river mouth and 256 m downstream of the uppermost HR station 9A. Milford WWTP effluent discharge point was located approximately 150 m upstream of river sampling station 5. The tidal intrusion of saltwater in the HR is detectable at this location. Further downstream is the discharge location for the Stratford WWTP. The sixth WWTP sampled in this location (Beaverbrook-Milford) discharges into a salt marsh area at the mouth of the HR, the Charles E. Wheeler wildlife refuge area, located between stations 2 and 3.

Several sites in the Long Island Sound (LIS) were selected for gauging the reach of the HR plume into the Sound, and the continued transport of PFAAs into the estuary. The salinities at these locations were consistently high (27-27.6‰) unless the HR fresh water plume was detected (20-25‰).

4.3 Experimental

4.3.1 Sampling site and collection parameters

Samples of final effluent from the six WWTPs discharging into the lower HR were obtained one week and one day prior to the summer survey along the HR during low

flow hydrological conditions, July 17th and July 23rd 2012 (data given in Chapter 3). On July 24th, 2012, duplicate water samples were collected from 4 locations along the HR (stations 1-4, Figure 4.1) from on-board the research vessel “Lowell Weicker”, and from the shore at stations 5, 8 and 9 (Figure 4.1). Surface water samples were obtained from the region of the LIS near to the mouth of the HR from aboard the *R/V Lowell Weicker* on July 25th 2012 (Figure 4.2). Detailed information regarding the station locations and water quality parameters for the summer river low-flow hydrology survey is given in Table 4.1.

A second field survey was conducted June 2013 during a period of high river discharge. However, budgetary constraints due to a 50% reduction in research funding led to a modified field sampling plan. Upstream river waters, sampled at one location, HR station 8, and the final effluents from the 2 WWTPs located near the river mouth (Milford and Stratford) were sampled one week prior to and one day prior to sampling of the estuary surface waters. Samples of the HR estuary surface waters were sampled either side of the river plume at HR station 1/LIS 1P (Figures 4.1 and 4.2) and a second station (LIS 20P) located 1350 meters due east of LIS 1P (Table 4.2). Water sample collection site information and water quality parameters for the high river flow regime study are given in Table 4.2. All water samples were collecting in duplicates.

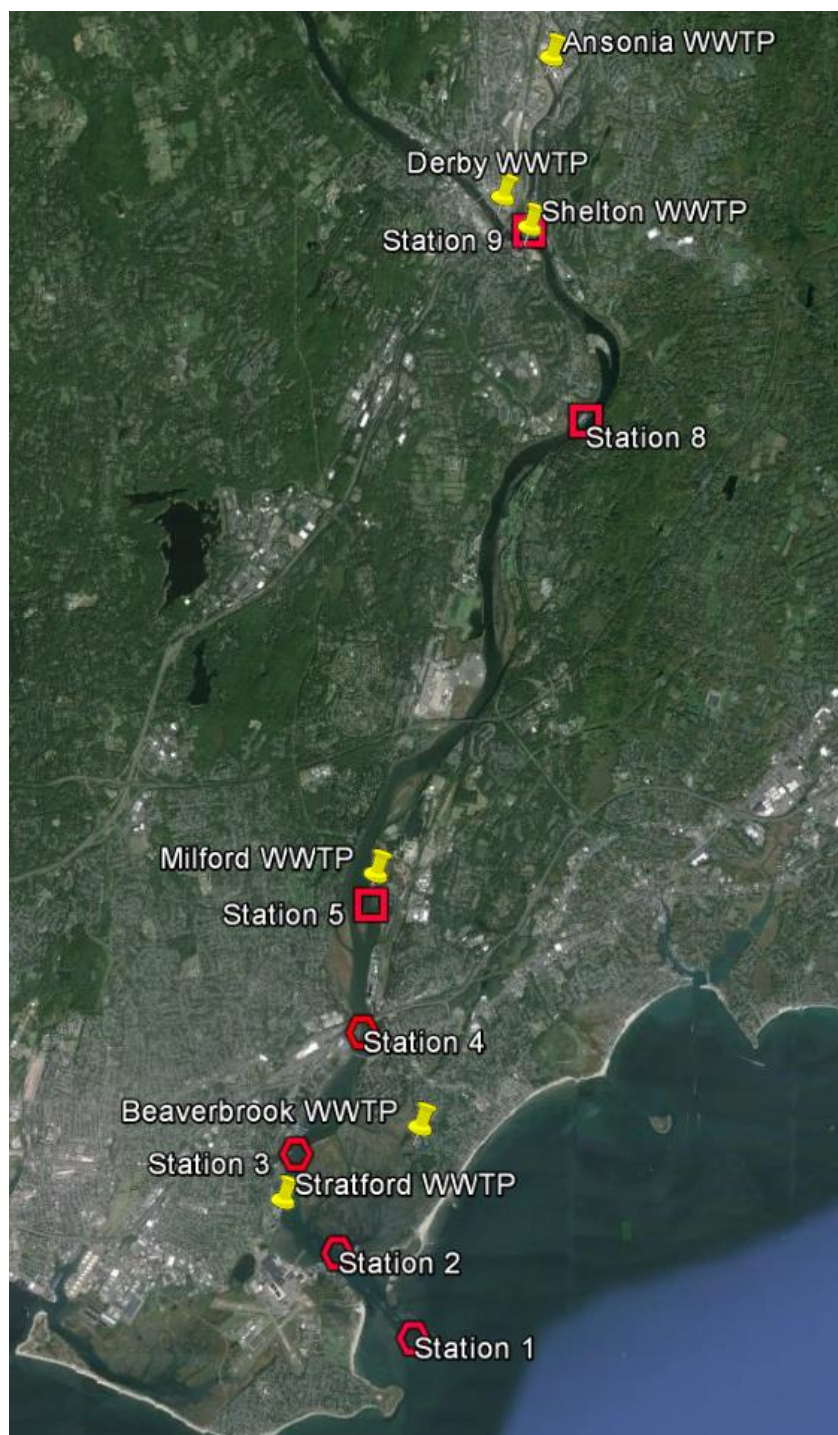


Figure 4.1: Map showing the locations of the sampling stations along the Housatonic River. Yellow pins show the WWTPs. Red markers indicate river sample sites; squares show where samples were collected from the shore, octagons samples collected from boat. Map from Google Earth.

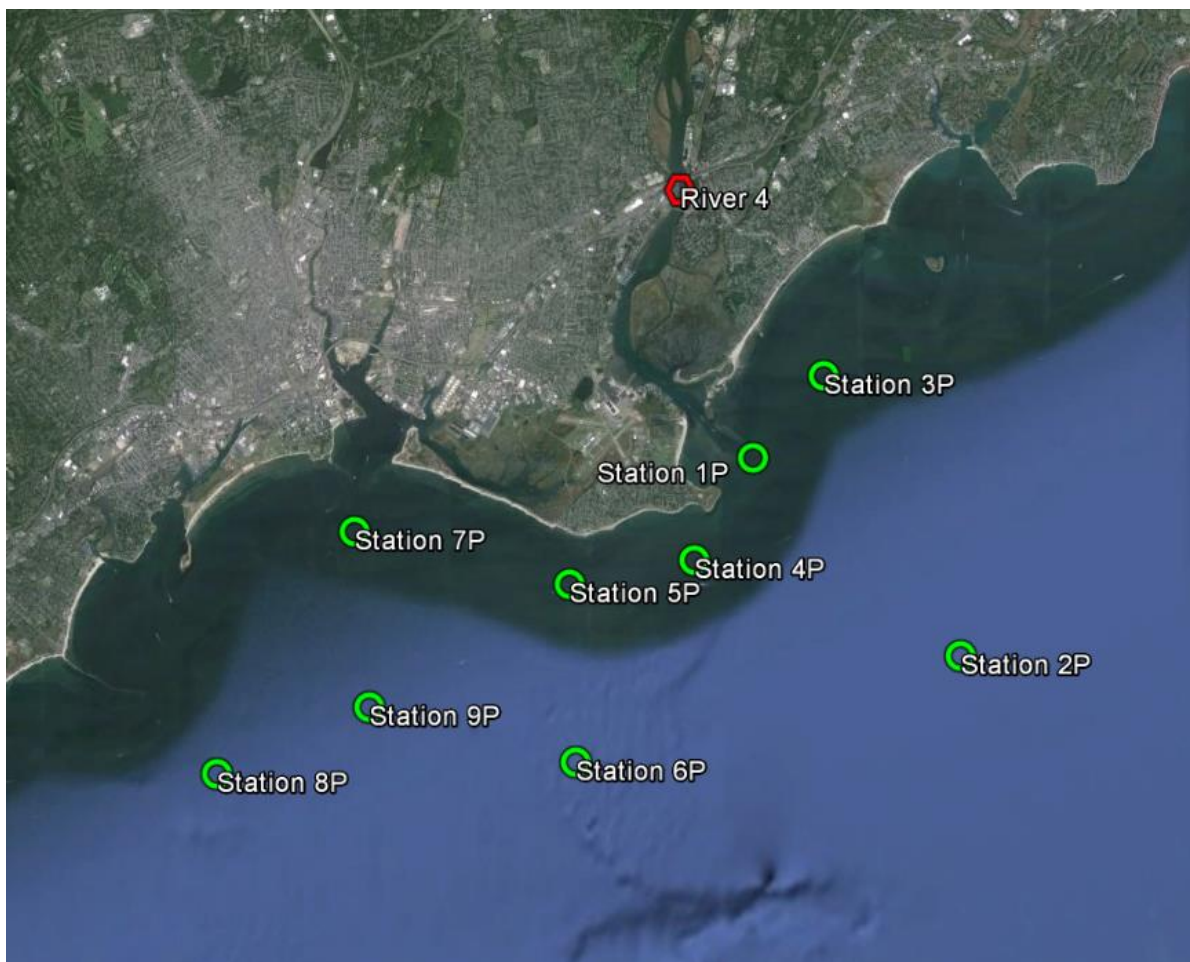


Figure 4.2: Map of sampling stations in the LIS.). Sampling stations in the LIS were given the suffix p (for plume) to distinguish between the river stations. (Map from Google Earth).

Table 4.1: Sampling station information and collection parameters for the summer low flow survey along the HR and regional LIS. nm=not measured.

Station	Location	Date	Time	Tide (time)	Salinity (psu)	Water temp °C	pH	Samples obtained
HR 1	41°09'35" N 73°06'05" W	7/24/12	15:50	High tide (15:32)	27.53/ 27.59	22.66	7.94	Surface/deep water Sediment grab
HR 2	41°10'23" N 73°06'48" W	7/24/12	16:21	Ebb	27.49/ 27.53	22.77	7.76	Surface/deep water Sediment grab
HR 3	41°11'11" N 73°07'18" W	7/24/12	16:54	Ebb	27.22/ 27.27	22.31	7.67	Surface/deep water Sediment grab
HR4	41°12'12" N 73°06'35" W	7/24/12	13:12	Flood	22.92	23.33	7.60	Surface/deep water Sediment grab
HR 5	41°13'15" N 73°06'32" W	7/24/12	18:24	Ebb	11.27	25.47	nm	Surface water
HR 8	41°17'17" N 73°04'13" W	7/24/12	19:59	Ebb	0.35	25.73	nm	Surface water
HR 9A	41°18'53" N 73°05'08" W	7/24/12	19:05	Ebb	0.13	26.20	nm	Surface water
HR 9B	41°18'53" N 73°04'54" W	7/24/12	19:20	Ebb	0.13	nm	nm	Surface water
HR4	41°09'35" N 73°06'05" W	7/25/12	08:32	Ebb	7.991	24.65	7.48	Surface water
LIS1p/HR1	41°09'35" N 73°06'05" W	7/25/12	09:12	Ebb	20.02	nm	7.48	Surface water Sediment grab
LIS 2p	41°07'42" N 73°02'56" W	7/25/12	10:14	Low tide (10:06)	27.49	22.34	7.98	Surface water
LIS 3p	41°10'29" N 73°04'42" W	7/25/12	09:31	Low tide	27.66	21.51	7.70	Surface water Sediment grab
LIS 4p	41°08'40" N 73°06'23" W	7/25/12	10:49	Flood	26.68	21.78	7.60	Surface water Sediment grab
LIS 5p	41°08'25" N 73°08'01" W	7/25/12	11:04	Flood	27.09	nm	7.66	Surface water
LIS 6p	41°06'42" N 73°07'54" W	7/25/12	11:24	Flood	27.20	nm	7.67	Surface water
LIS 7p	41°08'57" N 73°10'49" W	7/25/12	12:02	Flood	27.22	21.79	7.73	Surface water
LIS 8p	41°06'33" N 73°12'34" W	7/25/12	12:38	Flood	27.27	nm	7.65	Surface water
LIS 9p	41°07'12" N 73°10'35" W	7/25/12	12:54	Flood	27.12	21.94	7.73	Surface water
LIS1p/HR1	41°09'35" N 73°06'05" W	7/25/12	14:00	Flood	27.67	21.88	7.76	Surface water

Finally, during low flow hydrology in October 2014, additional samples were collected from a location on the HR upstream from station 9 and above the Derby-

Shelton dam and from outside the effluent discharge points of the Derby and Milford WWTPs in order to further investigate upriver sources of PFAAs and loss to sediments in the proximity of the effluent discharge zone under different salinities (Derby 0 ppt and Milford 9.0 ppt).

Table 4.2: Collection locations and water quality parameters for survey conducted during high river discharge, June 2013. NR=Not recorded.

Sample location	Location	Flow rate (L/s)	Date	Temperature	pH	Salinity (ppt)
Milford WWTP	41°13'22" N 73°06'28" W	245	05/30/2013	19.0	6.3	0
Stratford WWTP	41°10'42" N 73°07'27" W	282	05/30/2013	21.5	6.64	0
HR station 8	41°17'17" N 73°04'13" W	220,000	05/30/2013	NR	NR	0
Milford WWTP	41°13'22" N 73°06'28" W	232	06/04/2013	20.0	6.4	0
Stratford WWTP	41°10'42" N 73°07'27" W	309	06/04/2013	21.4	6.64	0
HR station 8	41°17'17" N 73°04'13" W	220,000	06/04/2013	NR	NR	0
HR 1/LIS 1P	41°09'35" N 73°06'05" W	Unk	06/05/2013	14-1-17.7	7.8-8.1	10.5-21
LIS 20P	41°09'49" N 73°04'45" W	Unk	06/05/2013	14.1-17.7	7.8-8.1	18-27

4.3.2 Chemicals and equipment

For the survey conducted July 2012, PFAA analytical standards were purchased from Waters Corporation (Milford, MA) and included PFAA calibration standards (11 PFAAs (C₄-C₁₄) and 5 PFSAAs (C₄, C₆-C₈, C₁₀), PFAA mass labeled recovery standards (Perfluorohexanoic acid (1,2-¹³C₂), Perfluoro-n-[1,2-¹³C₂]-decanoic acid and

Perfluorohexanesulfonic acid ($^{18}\text{O}_2$) and PFAA internal standards (1,2,3,4- $^{13}\text{C}_4$ PFOA and 1,2,3,4- $^{13}\text{C}_4$ PFOS). A complete description of each target analyte is given in Table A1. For all other investigations, including the sampling campaign spring 2013, and the additional sampling October 2014, PFAA analytical standards used were obtained from Wellington Laboratories Inc., (Ontario, Canada); a mix of mass labeled isotopes (MPFAC-MXA) of PFCA and PFSA ($^{13}\text{C}_2$ labeled $\text{C}_{4,6,8-12}$ PFCAs and $^{13}\text{C}_2$ labeled $\text{C}_{6,8}$ PFSA) were used for recovery standards. A mix of native PFCAs ($\text{C}_4\text{-C}_{14}$) and PFSA ($\text{C}_{4,6,8,10}$) used for salinity water-SPM partitioning experiments. A complete description of the recovery standards from Wellington Labs can be seen in the Appendix Table A9.

Methanol (Optima LC/MS grade) ammonium hydroxide and ammonium acetate were purchased from Fisher Scientific. All equipment used was pre-cleaned by baking at 450°C for 4 hours minimum (glass) or thoroughly washed and rinsed with MilliQ and methanol (for non-glass components). All SPE tubing, valves and adapters were sonicated in methanol for a minimum of three 10 minute rinses.

4.3.3 Sample collection and extraction

The extraction method was initially validated using two volumes (0.5 L and 2 L) of ultrapure (MilliQ) water spike with 10 ng (absolute mass) of native PFAA mix, and 10 ng mass-labeled analogue (mPFAAs) recovery standards. Results are given in Appendix Figures A5, A6 and A7. SPE extraction recoveries of PFCAs $\text{C}_4\text{-C}_{11}$ and PFSA are within acceptable ranges (80% - 120%) and adjusting with recoveries using mass-

labeled recovery standards resulting generally in better recoveries with lower standard deviation.

Effluent samples from the WWTPs located along the HR were extracted and analyzed as previously described in Chapter 3, section 3.2.3. River surface water was collected in pre-cleaned (methanol and milliQ rinsed, as well as 3x sample rinses) 5 L polypropylene (PP) carboys from the side of the boat (river stations 1, 2, 3 and 4, and all LIS stations) or from shore (stations 5, 8, 9A and 9B). Deep water samples were collected 1 m from the bottom using go-flow bottles, and emptied into 5 L polypropylene (PP) carboys. Sediment samples were obtained using a grab sampler. Samples were stored on ice until arrival at the laboratory, and then stored at 4 °C until filtration, which was performed within 48 hours of sample collection. Following filtration, the aqueous filtrate samples were stored at 4 °C until extraction, which was completed within 48 hours of filtration. Sediments samples and suspended particulate matter (SPM) collected on filters were frozen following collection. 4 L of sample was filtered 1 L at a time into 1 L PP sample containers using 0.45 µm nominal pore size PP pre-filters. All PP filter membranes (4L filtration) were collated into one sample for extraction of SPM. Filtrate samples were divided into two samples of 2 L for WAX SPE extraction.

Suspended particulate matter (SPM) extraction: The PP filters were placed into pre-cleaned 15 mL PP centrifuge tubes, 10 ng of PFAA recovery standard mix was spiked directly onto the a filter, and 12 mL methanol added. Tubes were refrigerated for a 24 hour extraction, followed by sonication for 1 hour. The extract was then decanted into a second PP centrifuge tube and stored at 4°C, while the filters were extracted a second time in methanol for 24 hours at 4°C followed by sonication. Both methanol extracts

were combined and reduced under a gentle stream of ultra-pure nitrogen to a final volume of 0.5 mL. The final extracts were then cleaned by a method previously reported (33, 34) 50 mg of ENVI-carb (Supelco) was weighed into a 1.5 mL PP centrifuge tube, and triple rinsed by vortexing in methanol. The extract was added to the ENVI-carb, vortexed for 10 seconds, centrifuged, and then transferred to a clean 1.5 mL PP vial.

Aqueous phase extraction: 2 L samples of filtrate were spiked with 10 ng of the PFAA recovery standard mix for solid phase extraction (SPE) using a method previously described (5). Oasis WAX Plus (225 mg) SPE cartridges (Waters Corporation, Milford, MA) were mounted on a Miniprep® vacuum manifold, with all poly(tetrafluoroethylene) (PTFE) materials removed and replaced by PP counterparts to avoid any potential fluoropolymer contamination. Extraction cartridges were pre-cleaned with 10 mL methanol with 0.1% ammonium hydroxide, followed by 5 mL methanol, and conditioned with 5 mL of MilliQ water. Samples were extracted at a flow rate of approximately 50 μ L/second, washed with 10 mL of 10% methanol in MilliQ water and dried under vacuum for 20 minutes before elution with 6 mL of methanol with 0.1% ammonium hydroxide followed by a second elution with 6 mL methanol. Elutes were concentrated under a gentle stream of ultra-high purity nitrogen to a final volume of 0.5 mL.

Sediment extraction: Sediment samples were lyophilized, and 5 g of dried sediments was taken from the center of each sample tube and placed in a pre-cleaned (methanol rinsed) 50 mL PP centrifuge tube. 10 ng PFAA recovery standard spiking mix was added and samples were extracted with two 24 hour soaks in 30 mL of methanol at 4 °C followed by 1 hour sonication (as described for filter extractions). Extracts were then collated and reduced under nitrogen to a final volume of 0.5 mL. The final extract was

cleaned using ENVI-carb as described for SPM extraction. Prior to instrumental analysis, all extracts were filtered with a 0.2 μm nylon syringe filter (pre-rinsed with methanol) into a 300 μL PP autosampler vial with PP screw cap.

Oyster extraction: Oysters were deployed in mesh net bags, attached to two docks in near surface waters, at Brewers Marina located near HR station 3, and at Knapp's landing, Stratford, opposite HR station 2, for ten days beginning just prior to the HR field study, July 17th, and collected July 27th 2012. Additional oysters were collected from river bed at HR station 2 by grab sampling. Oysters were extracted using a method previously published (35). Oysters were collected in duplicates for each location, stored in a 50 mL PP centrifuge tube, and frozen prior to extraction. Whole oysters were removed from the shell and placed into pre-cleaned 50 mL PP centrifuge tubes and lyophilized. 30 mL of 0.01 N KOH/methanol was added to each whole oyster sample, and samples were then homogenized by a mechanical homogenizer, which was thoroughly washed prior to and between uses with a sequence of MilliQ ultra-pure water and methanol. 10 ng (absolute mass) of PFAA recovery standard mix (Waters Corp, MA) was added to each oyster sample at the mixture was shaken at 250 rpm at room temperature for 16 h. After digestion, the tissue solution mixture was centrifuged, and the supernatant added to a 500 mL PP bottle containing 450 mL of ultra-pure water. The oyster tissue was rinse with a second aliquot of 20 mL 0.01 N KOH/methanol, vortexed and sonicated for 2 hours, followed by centrifuging. The second supernatant was added to the first, and the solution then extracted using WAX SPM columns as described above for aqueous phase extraction.

4.3.4 Instrumental analysis and quantitation

Samples were analyzed for 16 target PFAAs (11 PFCAs- C₄-C₁₄, and 5 PFSAAs- C_{4,6-8,10}) on a Waters Acquity ultra-performance liquid chromatography system with triple quadrupole tandem mass spectrometer (UPLC-MS/MS) at the Center for Environmental Science and Engineering (CESE) at the University of Connecticut, retrofitted for PFC analysis using the PFC analysis kit from Waters Corporation. Instrumental analysis parameters were as described previously (36). Briefly, 8 μ L aliquot of sample was injected onto an Acquity BEH C18 column (2.1 μ m x 50 mm; Waters Corp.) that was held at 50°C. Analytes were eluted using a gradient mobile phase of 2mM ammonium acetate buffer in methanol at a flow rate of 500 μ L/min. Electrospray negative ionization (ESI) was used and the mass spectrometer was operated in multiple-reaction-monitoring (MRM) mode. Quantitation was performed using MassLynx software, with a linear 1/x weighted regression fit. Calibration curves were prepared from the methanolic standards in the range of 1-500 ng/mL and the instrumental detection limit was determined using a 3:1 signal to noise (S/N) ratio of the lowest concentration methanolic standard (Appendix Tables A10, A11 and A12). UPLC run parameters and MRM transitions monitored for PFAAs analyzed during the summer low flow survey July 2012 and for the oyster study are listed in Appendix (Tables A2 and A3). UPLC MRM transitions monitored for PFAAs analyzed for subsequent analysis in 2013 and 2014 are listed in Appendix Table A9. While the UPLC conditions did not change, the MRM transitions were increased to include more isotope labeled PFAA surrogate standards which replaced the previous use of 3 labeled PFAA recovery standards and 2 instrument standards with a suite of 9 labeled standards, added prior to sample

extraction and used to account for both recovery and instrument variability, as per a method previously published (36). Aqueous phase PFAA concentration analysis was performed using SPE extracted standard curve, whereas a serial dilution standard curve was used for solid phase extractions. Concentrations of the individual PFAA compounds were normalized against the recoveries of their mass-labeled counterparts which were added prior to extraction. For PFAA compounds without a mass-labeled counterpart, the neighboring mass-labeled compounds were used (Table A9). Samples from the high river-discharge survey were subject to re-analysis using a comparative assessment of serial dilution vs. SPE extracted standard curve (see Appendix Figure A8).

4.3.5 Quality control

Pre-cleaned PP sample bottles containing MilliQ were used as field blanks to evaluate contamination during sample transport and storage; reagent blanks were used to evaluate extraction contamination. Additional pre-cleaned sample bottles containing MilliQ were spiked with 5 ng (absolute mass) PFAA mixture or with a PFAA QC solution mix (4.3–25 ng) to evaluate analyte loss and extraction performance. Blank PP filters were also extracted; using PFAA spiked (10 ng) filters to evaluate extraction performance, and unspiked filters, to evaluate contamination throughout the extraction procedure. An ENVI-carb clean up blank was also performed, as well as a nylon-needle filter blank, to ascertain that these additional steps could yield any contamination. No PFAA target analytes were detected in any of the field, reagent, PP filter, or ENVI-carb clean up blanks above the instrument detection limit with the exception of several PFAAs detected in the field blank obtained from the WWTP survey May 30th 2013, at concentrations above MDL. Each result obtained for the 5/30/2013 survey was therefore

corrected by subtracting the concentration value obtained in the field blank from the sample concentration.

The limit of quantitation can vary from one analysis to another as it can dependent upon background. Since effluent, riverine and estuarine waters constitute different potential background effects, reporting limits were set as follows; limit of detection (LOD) and limit of quantitation (LOQ) were set at signal to noise ratio (S/N) >3 and S/N >10 respectively for each individual target analyte peak in each sample run. Data was therefore only deemed reportable for each individual peak with a S/N ratio >10. Reported concentrations were also corrected for potential ion suppression or enhancement using isotopically labeled recovery standards (Table A4 Survey 1, Table A10 all subsequent analysis). Instrument detection limits of each target PFAA detected was estimated by extrapolation from the smallest calibration standard (Appendix Table A11). Detailed information concerning the amount of mass-labeled recovery standards detected in the samples is given in the Appendix Tables A13 and A14.

4.3.6 Elemental analysis

Duplicate samples of river water (60-100 mL) were collected on board by syringe filtration through pre-combusted GF/F glass fiber filters (GFF), and grab samples of sediments were collected and stored in 50 mL PP centrifuge tubes. SPM collected on the GFFs was lyophilized and analyzed for total carbon, nitrogen, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on the Costech 4010 elemental analyzer (combustion/oxygen temperature 980 °C, reduction temperature 700 °C), connected to a Thermo Delta V Advantage continuous flow gas chromatography isotope-ratio mass spectrometer (IRMS) with a ConFlo IV interface, Analysis for carbon, nitrogen, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was performed according to the USGS

method code RSIL 1832 (37). The duplicate set of GF/F filters with SPM were acidified overnight prior to lyophilization and analysis for organic carbon content.

4.4 Results and Discussion

4.4.1. Summer low flow conditions

4.4.1.1 PFAAs in wastewater effluent

Samples of wastewater effluent were obtained from the 6 WWTPs located along the lower HR and estuary both one week prior to and one day prior to sampling the HR river, July 17th and July 23rd 2012. Concentrations of PFAAs detected in both dissolved phase (filtrate) and SPM (collected on 0.45 µm nominal pore size PP filters) phases were given in Chapter 3, section 3.3.1. Average (n=2) ΣPFAA (aqueous plus SPM phases) concentrations seen in the effluent samples are given in Table 4.3. The most commonly detected PFAAs were PFPA, PFHxA, PFHpA, PFOA, PFNA, PFHxS and PFOS, which were detected in 100% of samples on both sampling dates. PFPA and PFOA were the predominant PFCAs, with concentrations ranging from 11.2 – 72.5 ng/L and 14.6 - 45.2 ng/L respectively. PFOS was the highest measured PFSA, with concentrations ranging from 8.3 - 74.4 ng/L. Total overall PFAA concentrations for both weeks ranged from 96.5 ng/L – 335.1 ng/L. Four out of the six WWTPs concentrations of total PFAAs increased slightly in the second week. Of the other two, Derby, decreased in total PFAA concentrations, and Stratford had no change. Concentration ratio profiles however remained remarkably consistent over the course of one week for

5 out of the six WWTPs (Figure 4.3) (linear regression values $R^2 > 0.83$), with the exception of Beaverbrook WWTP, which had a 2 fold increase in PFOA and PFNA on the second week of sampling. The consistency in the concentration and composition profile likely reflects domestic influent origin as the predominant source in this region (Figure 4.4). Both Milford and Stratford plants, which serve the greatest population number, have consistently higher total PFAA concentrations than the smaller WWTPs at Shelton and Beaverbrook; however the total PFAAs are highest in Derby and Ansonia (241.1 – 335.1 ng/L and 206.2 – 233.7 ng/L respectively) on both sampling dates.

PFAA discharges from the WWTPs into the Housatonic River were estimated from the concentrations measured in the final effluent and the average daily flow rate. Although the total PFAA concentrations were highest in Derby and Ansonia, the overall aqueous environmental contributions from these plants was smaller due to their relatively lower effluent discharge flow rates (0.7 – 1.4 g/day and 1.0 – 1.2 g/day) compared to the average daily mass flux calculated for the two larger WWTPs of Milford and Stratford (2.5 – 4.0 g/day and 2.9 -3.3 g/day respectively). The 6 WWTPs had an average combined PFAA daily mass discharge to the lower Housatonic receiving waters of 8.9 – 11.4 g/day with the highest contribution coming from PFPA, accounting for 19% of the total mass flux, and PFOA and PFOS, each accounting for 18% of the total mass flux.

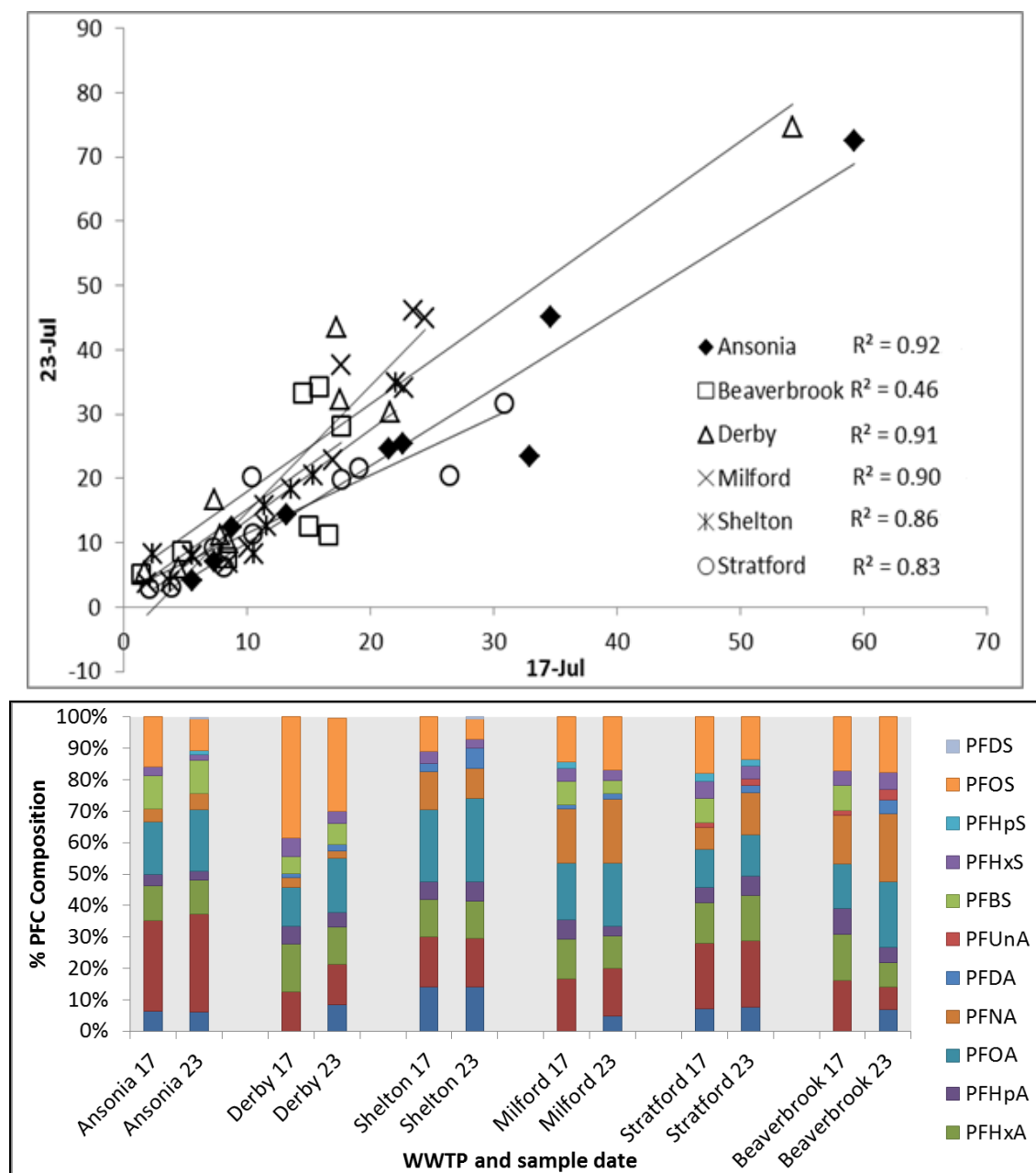


Figure 4.3: Comparisons of the PFAA concentrations (ng/L) in the final effluent samples collected one week apart from the 6 WWTPs located along the Housatonic River. Top: Comparative congener ratios between sampling weeks. Bottom: Comparison of congener profiles. Beaverbrook saw a 2x increase in the concentrations of PFOA and PFNA in the second week of sampling.

Table 4.3: WWTP effluent survey data- Σ PFAA (dissolved plus particulate fractions) concentrations (ng/L) [average \pm (range)] measured in final effluent obtained from 6 WWTPs along the Housatonic River, July 17th and July 23rd 2012. <LOQ= No peak with S/N >10. All values given have S/N >10. Values with no range indicate an n=1.

July 17th

WWTP	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFBS	PFHxS	PFHpS	PFOS	PFDS
Ansonia	13.2 (1.4)	59.3 (3.9)	22.7 (1.5)	7.4 (1.0)	34.7 (5.7)	8.8 (2.0)	<LOQ	<LOQ	21.5 (5.2)	5.6 (2.7)	<LOQ	33.0 (4.3)	<LOQ
Beaverbrook	<LOQ	16.6 (0.8)	15 (0.7)	8.4 (0.8)	14.6 (2.4)	15.9 (2.0)	<LOQ	1.4 (0.0)	8.2 (0.4)	4.7 (0.6)	<LOQ	17.7 (0.3)	<LOQ
Derby	<LOQ	17.6 (1.2)	21.6 (2.3)	7.9 (0.7)	17.3 (1.3)	4.5 (0.1)	1.7 (0.0)	<LOQ	7.4 (1.2)	8.4 (1.5)	<LOQ	54.3 (0.2)	<LOQ
Milford	<LOQ	22.8 (0.4)	17.0 (2.0)	8.5 (0.7)	24.5 (5.5)	23.5 (0.9)	1.9 (0.0)	<LOQ	10.1 (1.5)	5.6 (0.6)	2.8 (0.3)	19.6 (2.5)	<LOQ
Shelton	13.6 (4.3)	15.4 (3.9)	11.4 (3.4)	5.6 (0.9)	22.1 (7.1)	11.6 (2.9)	2.4 (0.0)	<LOQ	<LOQ	3.8 (0.6)	<LOQ	10.6 (0.6)	<LOQ
Stratford	10.5 (1.3)	30.9 (0.2)	19.1 (0.8)	7.3 (0.5)	17.7 (2.2)	10.4 (2.2)	<LOQ	2.1 (0.3)	11.5 (4.6)	8.1 (0.4)	3.9 (0.3)	26.4 (3.1)	<LOQ

July 23rd

WWTP	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFBS	PFHxS	PFHpS	PFOS	PFDS
Ansonia	14.3 (0.8)	72.5 (2.6)	25.4 (0.7)	7.0 (0.6)	45.2 (2.5)	12.4 (0.3)	0.5	<LOQ	24.7 (8.3)	4.2 (0.6)	2.8 (0.0)	23.4 (1.2)	1.3
Beaverbrook	11.1 (1.7)	11.2 (0.4)	12.6 (0.8)	7.6 (0.6)	33.2 (0.2)	34.3 (1.5)	7.2	5.1 (0.4)	<LOQ	8.6 (1.2)	<LOQ	28.2 (0.8)	<LOQ
Derby	21.2 (1.0)	32.3 (0.1)	30.4 (0.2)	11.2 (1.4)	43.5 (0.8)	6.0	5.4 (0.9)	<LOQ	16.7 (2.0)	10.1 (0.8)	<LOQ	74.7 (2.3)	0.8
Milford	11.0 (0.9)	34.0 (3.2)	22.9 (4.1)	6.9 (0.1)	44.9 (3.1)	46.1 (8.6)	3.7	<LOQ	9.3 (2.1)	7.8 (0.8)	<LOQ	37.7 (5.6)	<LOQ
Shelton	18.4 (1.4)	20.4 (0.8)	15.7 (0.1)	8.0 (1.0)	34.8 (6.5)	12.5 (0.8)	8.3 (0.2)	<LOQ	<LOQ	3.9 (0.3)	<LOQ	8.3 (0.3)	1.0
Stratford	11.5 (1.9)	31.8 (0.4)	21.6 (1.6)	9.3 (0.5)	19.8 (0.1)	20.2 (1.4)	3.5	2.9 (0.5)	<LOQ	6.3 (1.5)	3.2	20.4 (0.1)	<LOQ

4.4.1.2 PFAA concentrations in river waters

The field survey conducted in the summer of 2012 along the Housatonic River and estuary, as well as the two WWTP surveys conducted prior to, were during a time of no precipitation, therefore no samples were influenced by rain waters. The surface water samples obtained from the Housatonic River showed Σ PFAA concentrations ranging from 10.4 ng/L at the mouth of the river where it meets the LIS estuary (station 1, Figure 4.1) to 30.1 ng/L at the most up river sampling site on the Housatonic (station 9A) and to 77.7 ng/L at the mouth of the Naugatuck where it meets the Housatonic River at station 9B. At all 8 river sampling stations, eight of the 16 PFAA target compounds were detected ($C_5 - C_9$ PFCAs and $C_{4,6,8}$ PFSA) (Table 4.4). PFBA, PFDA, PFUnA, PFHpS and PFDS were detected sporadically. In almost all surface river water samples PFOS was the predominant PFAA, with concentrations ranging from 10.3 ng/L up-river to 2.4 ng/L at the river mouth. PFOA and PFPA were the predominant PFCAs, mirroring the predominance of these PFCAs in the WWTP final effluent, with concentrations ranging from 4.7 ng/L in the upper Housatonic (9A) and 17.8 ng/L in the Naugatuck (9B) to 1.4 ng/L at the river mouth (station 1) for PFOA, and 5.4 ng/L at 9A and 13.2 ng/L at 9B, to 1.7 ng/L at the river mouth for PFPA. Concentrations given are for the sum of dissolved plus particulate bound PFAAs. PFOS was the only compound detected associated with the suspended particulate phase in river and estuary water samples.

Ahrens *et al.* (2) reported similar concentration ranges in the River Elbe (Germany), with Σ PFAAs ranging from 7.6 ng/L, measured at the river mouth, to 26.4 ng/L at the up river site in Hamburg City. In contrast however, PFOA was the

predominant PFAA measured in the river water with concentrations ranging from 2.8 ng/L to 9.6 ng/L. A brief summary of several reported PFAA concentration values reported in river waters is given in Table 4.5 for comparison. In general, the concentrations observed in the surface waters of the HR are comparable to or lower than those reported in China, Japan, Europe, and in the US. In addition, the concentrations of individual PFAA species, such as PFOA, PFNA and PFOS, are more often reported in much higher concentrations than those observed in the HR, including values of PFOA more than 30x higher in the River Po, Europe, downstream of a fluoropolymer manufacturing plant (18), nearly 40x in receiving waters in the Conasauga River, Georgia, located in the proximity of the largest carpet manufacturing city in the world (Dalton, GA) (14), and 20x higher in a more local river, the Hudson, in NY state (38).

The highest concentrations of PFAAs were measured at the upper most-river stations, 9A on the main Housatonic, and 9B, at the mouth of the Naugatuck River, approximately 5-10 meters before it meets the Housatonic. The significance of the contributions of the WWTP discharge to the PFAA concentrations observed in river surface waters were calculated by comparing the mass flux from the nearest WWTP discharge (PFAA concentration (ng/L) effluent discharge rate (L/s)), which, in the case of station 9A was the Derby WWTP, and for 9B, the Ansonia WWTP.

Table 4.4: Concentrations of PFAAs and relative abundances (based on average concentrations) for samples of Housatonic River surface waters, stations 1 to 9A and 9B, in ng/L. (nd=not detected, SD= standard deviation).

PFAA	Frequency of detection (%)	Concentration range	Concentration mean \pm SD	Relative abundance (%)
PFBA	37.5	<LOQ – 2.8	1.8 \pm 0.9	5.7
PFPA	100	1.1 – 13.2	4.3 \pm 4.0	14.2
PFHxA	100	0.8 – 10.7	3.4 \pm 3.3	10.9
PFHpA	100	0.7 – 8.0	2.7 \pm 2.4	8.6
PFOA	100	1.4 – 17.8	5.6 \pm 5.1	17.2
PFNA	100	0.9 – 6.4	2.5 \pm 1.8	7.8
PFDA	37.5	<LOQ – 2.2	1.4 \pm 0.8	3.5
PFUnA	12.5	<LOQ – 0.4	0.3 \pm 0.1	0.7
PFDoA	0	<LOQ	Nd	Nd
PFBS	100	1.1 – 2.5	1.7 \pm 0.6	5.1
PFHxS	100	0.6 - 2.8	1.6 \pm 0.9	5.0
PFHpS	75	<LOQ – 0.9	0.7 \pm 0.1	2.2
PFOS	100	2.4 – 12.1	6.1 \pm 3.5	18.1
PFDS	12.5	<LOQ – 0.3	0.3	0.9

Table 4.5: Brief summary of concentrations of PFAAs detected in river surface water samples reported in the literature (in ng/L)

Values given are either reported min-max values, or mean. (nd= not detected; NR=not reported).

Location	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	Ref.
River Elbe, Germany	NR	0.9-3.1	1.6-5.0	0.8-2.4	2.8-9.6	0.2-1.1	<LOD-0.7	<LOD-3.4	<LOD-1.3	0.6-2.9	(10)
River Seine, France	NR	2.3-13.7	3.0-16.0	0.5-5.5	1.1-18.0	0.1-1.2	0.1-1.0	0.6-2.6	3.9-12.0	9.9-39.7	(28)
Glatt River, Switzerland	NR	NR	nd	0.7 – 2.7	7.0 – 7.7	nd	nd	2.3 – 7.7	8.9 – 14	43 - 60	(8)
Georgia, USA	NR	NR	NR	NR	3.1 - 480	<0.6 - 369	<0.1 - 131	NR	NR	1.0 - 318	(14)
Hanjiang River, China	NR	NR	NR	NR	81.0	18.9	27.8	NR	NR	51.8	(39)
Tsurumi River, Japan	NR	NR	5.5	3.1	15.9	38.0	3.9	NR	NR	179.9	(29)
Liao River, China	NR	NR	NR	nd – 23.3	nd – 27.9	nd	nd	nd	1.4 – 94.5	nd – 6.6	(23)
River Po, Italy	NR	NR	19	6.6	200	1.46	NR	NR	NR	NR	(18)
Danube, central Europe	NR	NR	3.0	0.95	16.4	0.27	NR	NR	NR	NR	(18)
Seine, France	NR	NR	13.3	3.7	8.9	1.26	NR	NR	NR	NR	(18)
Loire, France	NR	NR	3.4	0.9	3.4	0.43	NR	NR	NR	NR	(18)
Thames, UK	NR	NR	32	4.1	23	0.79	NR	NR	NR	NR	(18)
Rhine, Germany	NR	NR	18.2	1.8	11.6	0.55	NR	NR	NR	NR	(18)
Hudson, NY, USA	NR	NR	NR	NR	22 - 173	NR	NR	NR	0.7-1.6	1.5-3.4	(38)
Coyote Creek, CA	NR	NR	NR	nd	nd-15	nd	nd	NR	<1	27-38	(40)
Minnesota, US	NR	NR	NR	0.7	1.2	1.9	NR	NR	NR	8.8	(41)

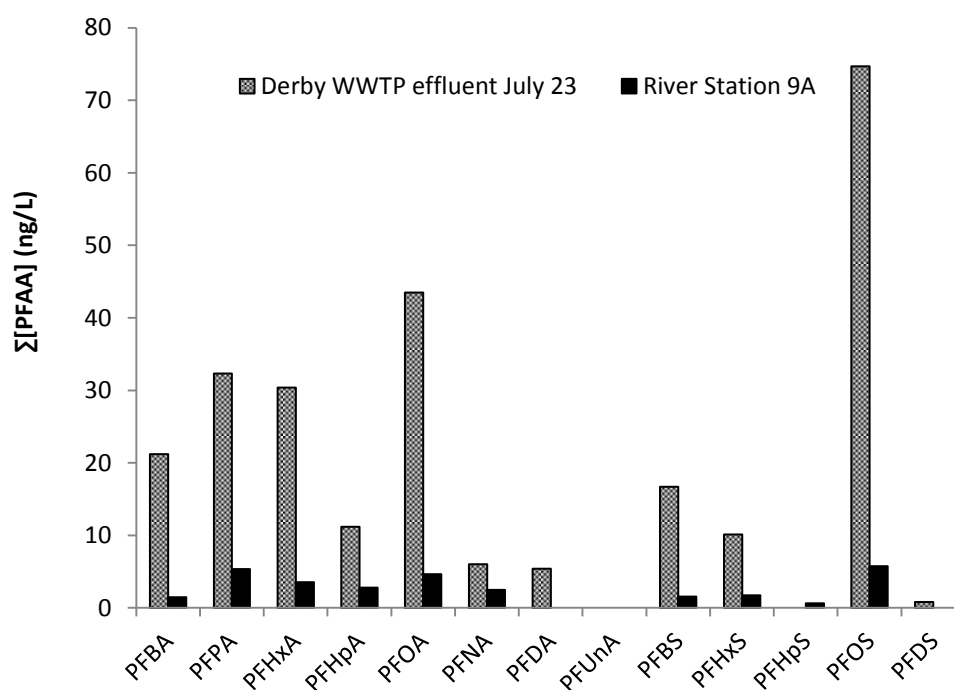
The distance between the Derby effluent pipeline and sampling station 9A was 280m (Figure 4.4A). Concentrations of PFAAs in surface water samples obtained at station 9A ranged from 2.4x (PFNA) to 13x (PFOS) lower than in effluent waters (Figure 4.4B); it is clear that WWTP effluent is a major source of PFFAs to the river. If we consider the volume flux of water at this section of the Housatonic main branch (as measured by USGS gage located at the Stevenson Dam, approximately 11 km upriver from the river sample station 9A), and the mixing of the Derby effluent discharge with this volume of water, the concentrations measured at 9A range from approximately 24x (PFBS) to 96x (PFOS) greater than would be predicted, indicating a major upriver source(s) of PFAAs to the HR. However at this summer's low-flow conditions it is possible that the sample obtained at station 9A was not well mixed with the full Housatonic body of water, therefore concentrations measured at 9A could reflect more of a direct sampling of the effluent plume. That being the case, the dilution factor for PFOA and PFOS, from effluent to surface water at 9A, was greater than for most of the smaller C-F chain PFAA species, which may indicate a potential loss of the longer chained species to the sediments in the close proximity of the effluent discharge zone. Becker *et al.* (42) previously reported concentrations of PFOA and PFOS in the sediments of the Roter Main River to be 3 and 40 times greater respectively, in the sediments downstream of WWTP discharge relative to river water concentrations.

The tertiary stage of the wastewater treatment process at the Derby WWTP is chlorination, during which the final effluent is held in a contact chamber prior to release. The effluent release therefore occurs at sporadic times; it is also possible that the sample obtained at station 9A did not reflect the contribution of the Derby WWTP

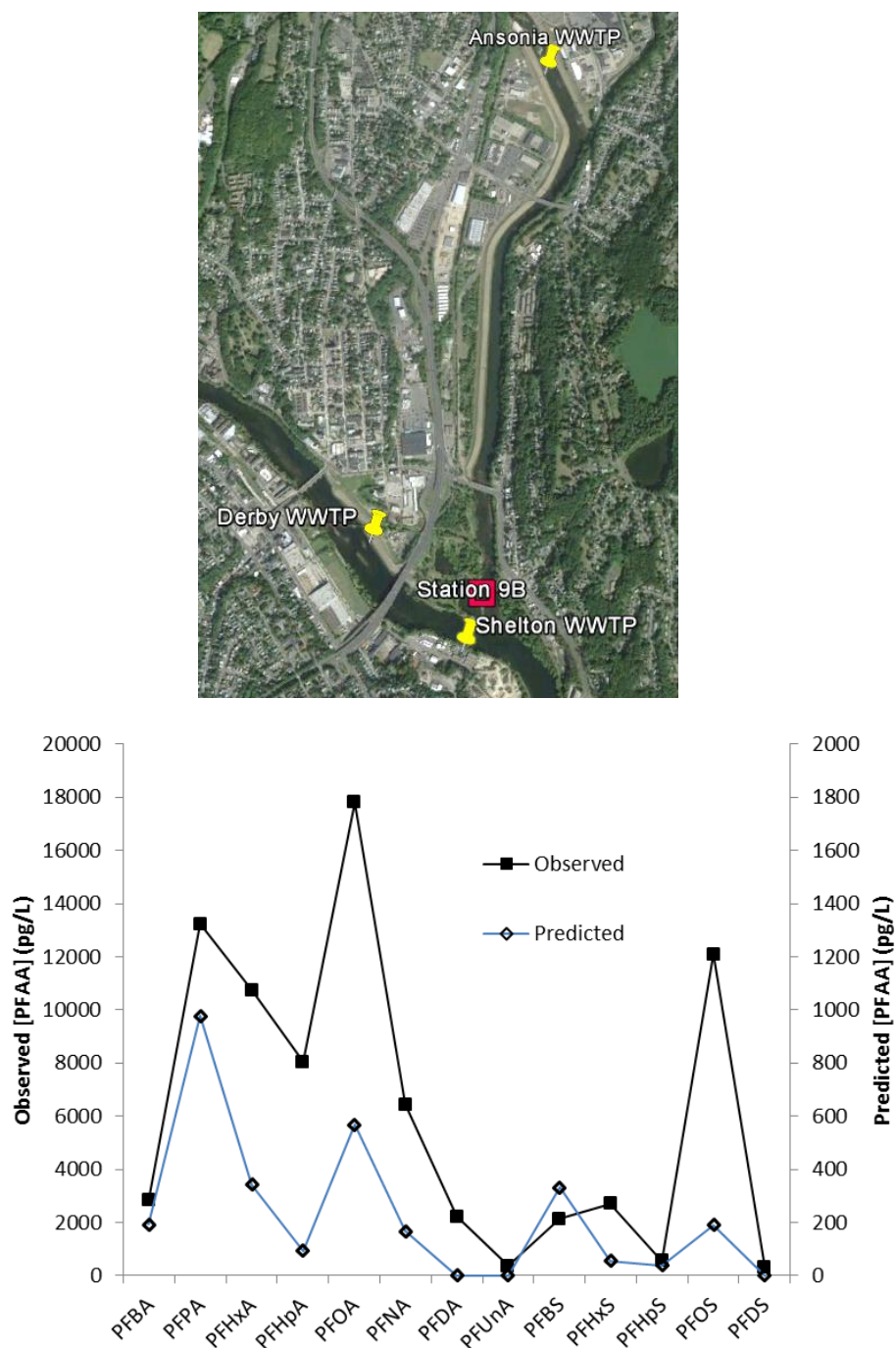
discharge at all, and the concentrations of PFAAs measured at 9A reflect upriver sources. Further investigations were deemed warranted in order to determine the contributions of upriver sources versus the input of the Derby WWTP at this sampling location on the Housatonic River, and to estimate the potential loss to sediments in the proximity of the effluent discharge zone.

The highest PFAA concentrations were measured in the Naugatuck River (station 9B). Effluent from the Ansonia WWTP discharges directly into the Naugatuck, and with a UV tertiary disinfecting process, effluent discharge from this plant is continuous (unlike that of Derby which has periodic releases following a chlorine disinfection stage). The contributions of the Ansonia WWTP discharge to the concentrations of PFAAs measure at station 9B were calculated, assuming the concentrations measured in the effluent to be constant.

The mass flux to station 9B was calculated using the volume flux from the Naugatuck measured by the USGS gauge located at Beacon Falls (4460 L/s), with the additional volume flux from the Ansonia WWTP (average daily flow rate). The distance between the Ansonia effluent discharge point and the sampling site 9B was 2,600m (Figure 4.5A), therefore complete mixing was assumed for the comparison of the expected vs. observed PFAA water concentrations at river sampling station 9B. Observed PFAA concentrations were found to be 10-60 times greater than expected based on the mass flux of PFAAs measured in Ansonia WWTP effluent (Figure 4.5B) also indicating the significance of upriver sources of PFAAs to the Naugatuck River, as observed in the Housatonic.



Figures 4.4 A and B: Concentrations of PFAAs measured in samples obtained from the upper Housatonic River (station 9A) compared to the concentrations of PFAAs measured in the final effluent of the Derby WWTP. **4.4 A:** (top) Map (google Earth) showing the proximity of the Derby WWTP effluent outfall to the river sampling station 9A. The distance between the two locations was 280 meters. **4.4 B:** (bottom) Concentrations of PFAAs measured in the dissolved phase of effluent and river water.



Figures 4.5 A and B: Sampling locations and PFAA concentrations in the Naugatuck River. **A (right):** Map showing the proximity of the Ansonia WWTP outfall to the sampling station 9B at the mouth of the Naugatuck River. The distance between the Ansonia outfall and the river station 9B was 2,600 meters. Map from Google Earth. **B (left):** PFAA concentrations observed in samples obtained at station 9B (Naugatuck River mouth) compared to the PFAA concentrations expected based on the PFAA mass loading from Ansonia WWTP and assumed complete mixing with the Naugatuck (concentrations in pg/L).

Seven WWTPs are located along the Naugatuck River, including Ansonia (Figure 4.6). Daily flow rates (MGD) for the WWTPs which discharge directly into the Naugatuck River, on the date of the field survey, ranged from 19.6 for the largest plant in Waterbury, 7.1 and 6.2 for the medium sized plants in Torrington and Naugatuck, to the lower flows from the remaining four smaller facilities, with flow rates of 1.6 (Ansonia) or less. Based on the daily flow rates from the 7 WWTPs located on the Naugatuck, and the Naugatuck River flow data from the USGS gage located in Beacon Falls, the calculated proportion of WWTP effluent making up the volume of the Naugatuck was estimated to be 35%.

WWTPs were assumed to be a major source of PFAAs to the Naugatuck. Using average PFAAA concentrations measured in the six lower Housatonic River WWTPs, the expected concentrations of PFAAs in the Naugatuck were calculated using the estimated 35% effluent composition in river waters. Under these assumptions, a better correlation was found between the expected (calculated) and the observed PFAA concentrations at 9B (Figure 4.7). These results indicate that 1- the assumption of WWTP derived loading being the major source to the Naugatuck River is good, and that 2- PFAA mass is conserved along the river, with no significant losses to sediments.

Based on these observations for the Naugatuck River, it is likely that the source of PFAAs to the upper Housatonic River system is from additional upriver WWTPs. Further investigations determined that there are several WWTPs on the upper Housatonic; New Milford (capacity of 1.02 MGD) and New Canaan (capacity 1.70 MGD) in CT, and two plants located in MA, Pittsfield (17 MGD capacity) and Great Barrington

(3.2 MGD capacity). In addition, there are a number of WWTPs are located along tributary rivers; Salisbury (0.67 MGD, Factory Brook), Newtown (0.93 MGD, Pootatuck River), Norfolk (0.35 MGD, Blackberry River), Southbury (0.78 MGD, Pompergaug River), Winchester (3.5 MGD, Still River) and finally a large WWTP located in Danbury (15.5 MGD capacity) located on the Still River. The mass fluxes from these additional WWTPs was estimated using the annual average daily discharge values from all WWTPs reporting to discharge into the Housatonic River and its tributaries in CT, MA and NY states. These values were obtained from the US EPA's Discharge Monitoring Report Pollutant Loading Tool, available online at <https://cfpub.epa.gov/dmr>. Combined discharged volumes for all upper Housatonic River WWTPs were compared to the volume flux of the Housatonic River, recovered at Stevenson Dam by the USGS, and available online at http://waterdata.usgs.gov/ct/nwis/uv?site_no=01205500. The calculated proportion of WWTP effluent making up the volume of the Naugatuck was estimated to be 7%. As previously done for the Naugatuck, the average PFAA concentrations previously measured in the six lower Housatonic River WWTPs was used to estimate the expected concentrations of PFAAs in the Housatonic River at Station 9A, using the estimated 7% effluent composition in river waters. The correlation between the expected (calculated) and the observed PFAA concentrations at 9A were not as strong as seen in the Naugatuck River model (Figure 4.8). The average differences between the predicted and observed PFAA concentrations in the upper Housatonic station 9A were an average of 6.6x greater (range of 3 – 24x greater) observed PFAA concentrations than would be predicted. In the Naugatuck River model, the average difference between observed and predicted PFAA concentrations was 1.3 x

(0.3- 2.7 range). The greater variability in the upper Housatonic model could indicate either WWTP point sources emitting greater PFAA concentrations than the average values assumed, important non-point sources, including runoff, atmospheric deposition, or in-situ formation of PFAA from precursor compounds, to the upper Housatonic.

The predicted PFAA concentrations for river sampling station 8, 4.5 km down river from the confluence of the Naugatuck and Housatonic rivers, was calculated 1- using the concentrations measured in the Derby WWTP effluent, mixing with Naugatuck (9B) and Shelton WWTP mass fluxes, and 2- using the concentrations of PFAA measured at stations 9A and 9B, mixing with Shelton WWTP waters, multiplied by the total volume flux of the rivers and WTP effluents. Comparison to the observed values resulted in better correlation ($R^2 = 0.68$) when using the mass flux from observed concentrations at 9A and 9B (approach 2), suggesting that the PFAAs measured at 9A were more likely to be from up river sources than a sampling from the Derby WWTP effluent plume (Figure 4.9).

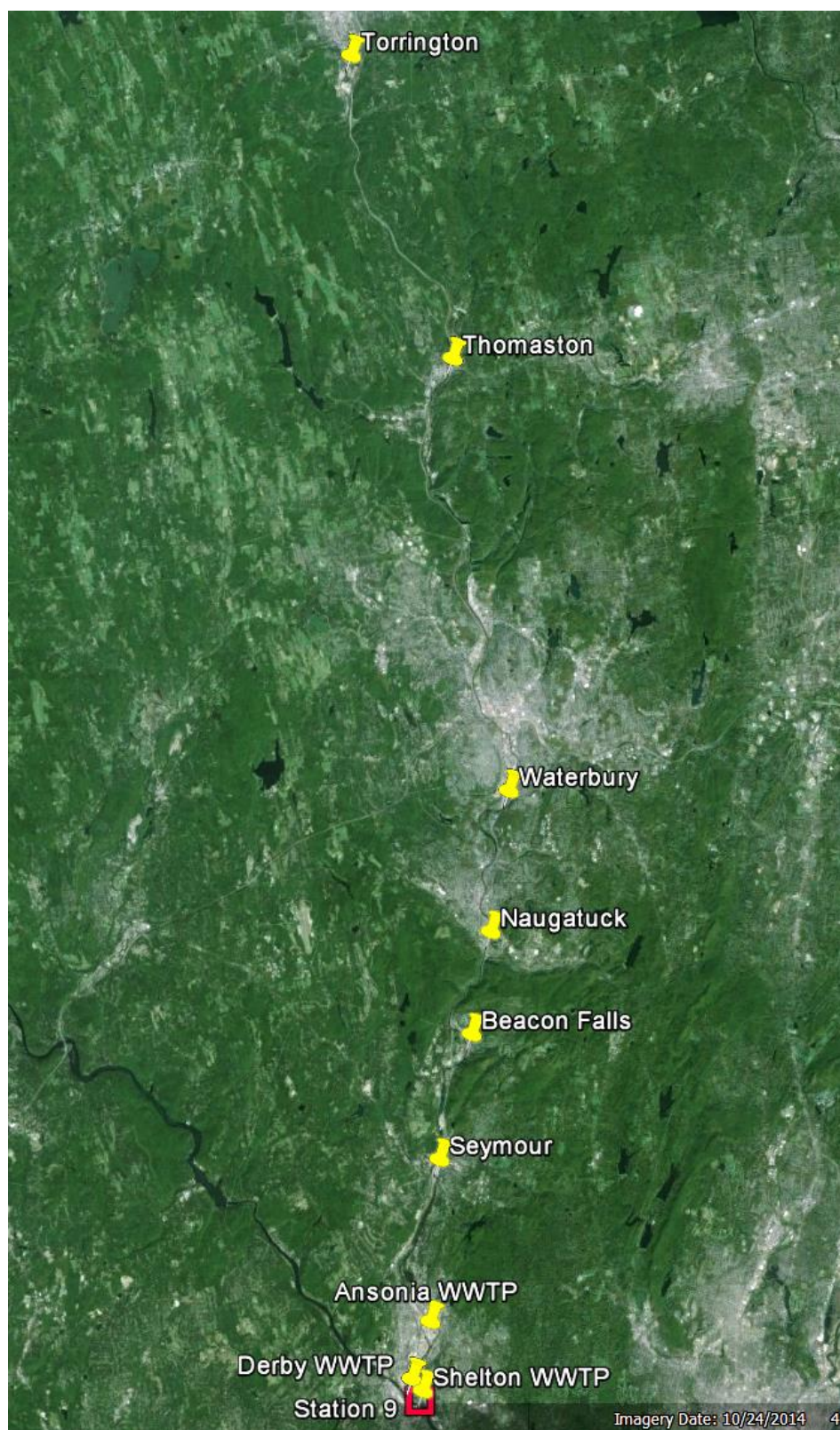


Figure 4.6: Locations of WWTPs located along the Naugatuck River. (Map from Google Earth).

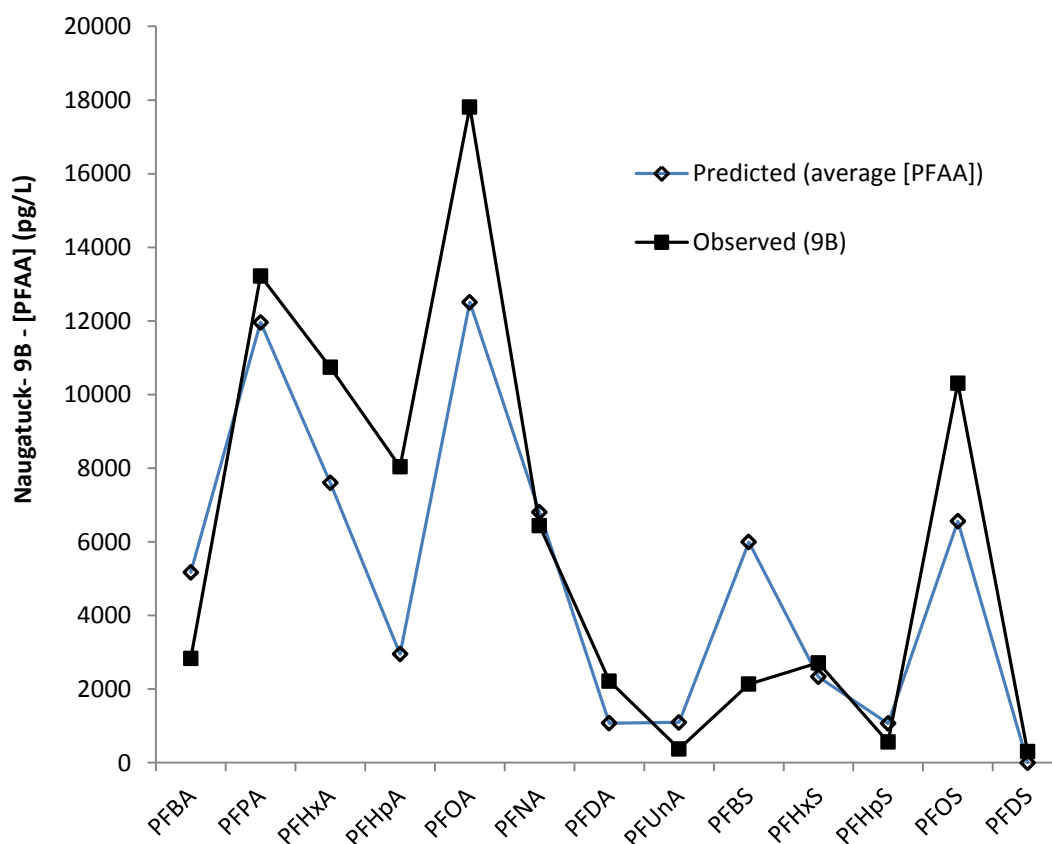


Figure 4.7: Concentrations of PFAAs measured from samples obtained at station 9B (Naugatuck river mouth) compared to concentrations of PFAAs expected assuming average PFAA concentrations measured in WWTP effluents in July 2012, and assuming the Naugatuck river is comprised of 35% effluent waters.

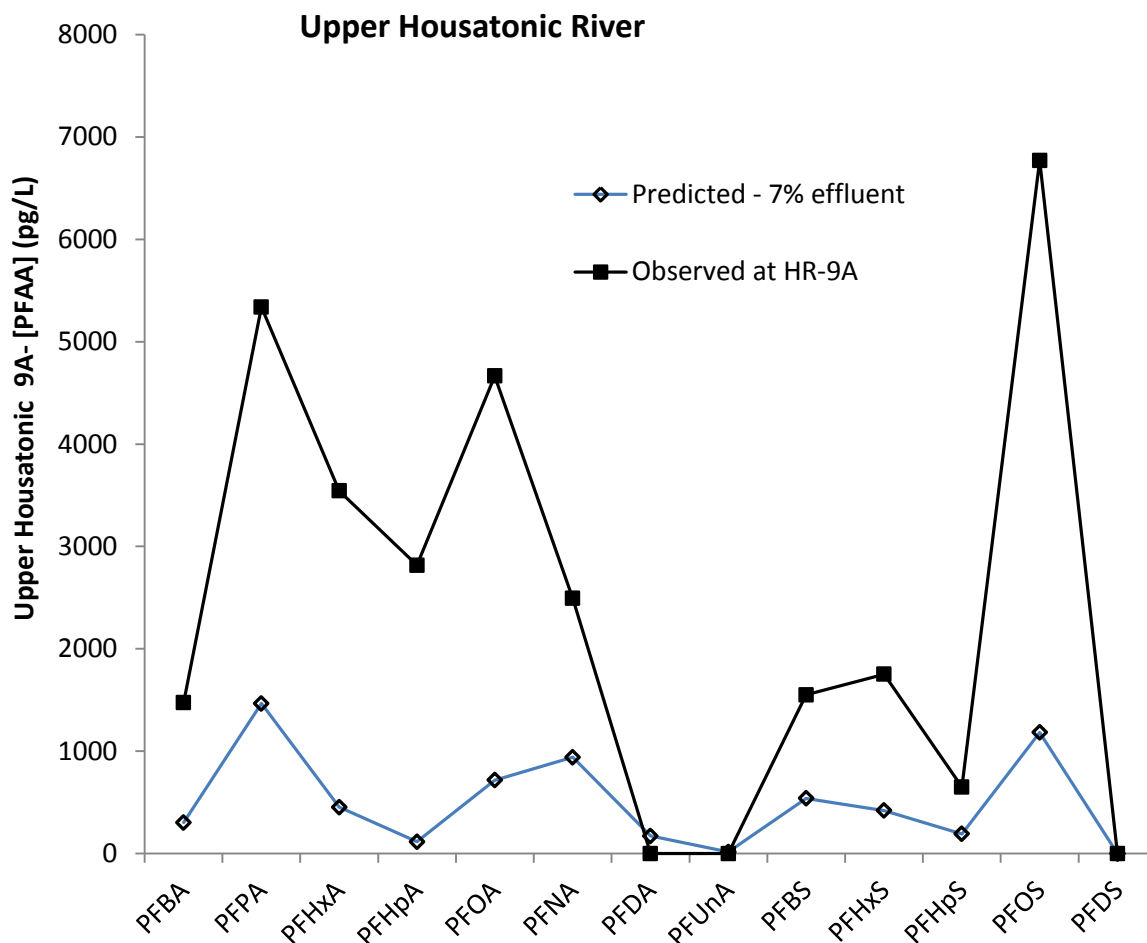
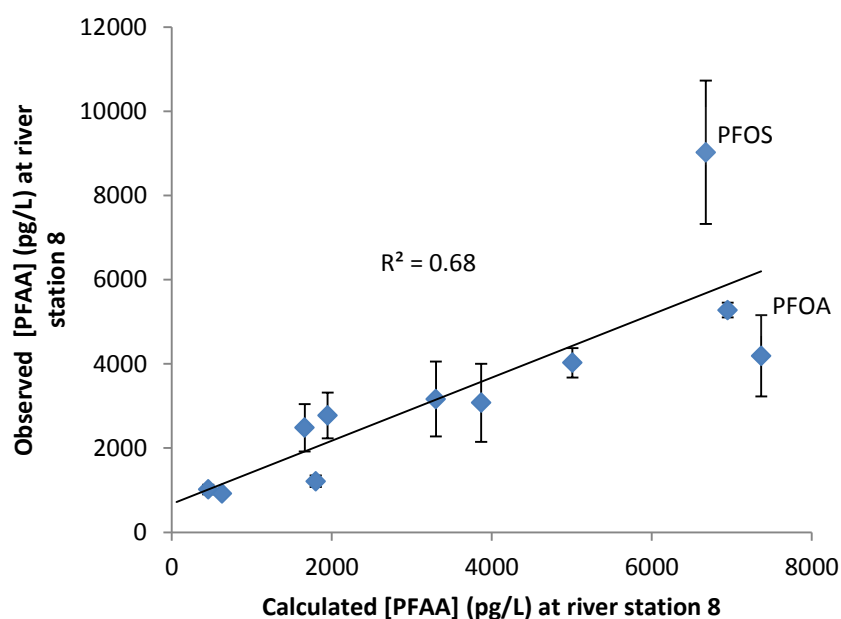
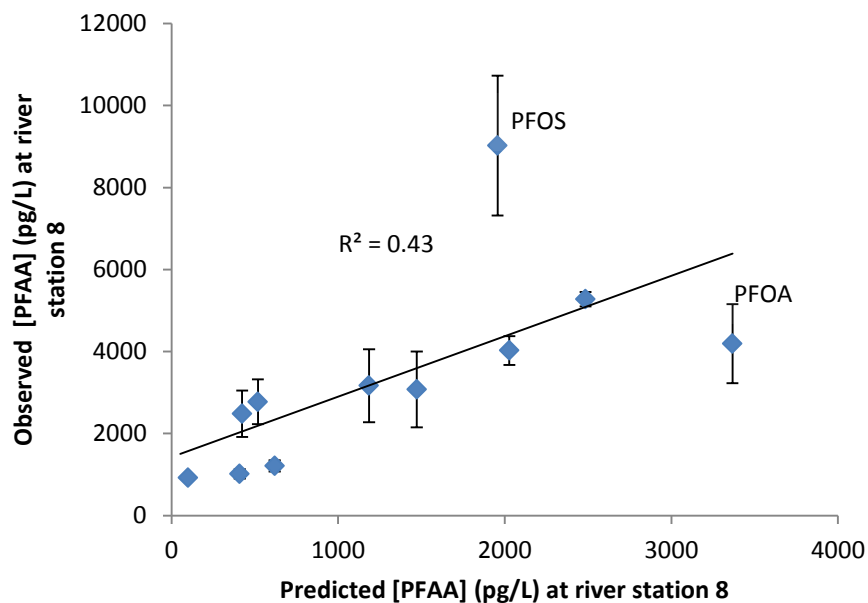


Figure 4.8: Concentrations of PFAAs measured from samples obtained at station 9A (upper Housatonic) compared to concentrations of PFAAs expected assuming average PFAA concentrations measured in WWTP effluents in July 2012, and assuming the upper Housatonic River is comprised of 7% effluent waters.



Figures 4.9 A and B: The expected PFC concentrations at station 8 were calculated using, **A** (top)- the sum mass flux from Derby and Shelton WWTPs plus the Naugatuck mass flux (station 9B); **B** (bottom)- calculated mass flux from observed PFAA concentrations at Stations 9A and 9B, plus the additional mass flux of PFAAs from the Shelton WWTP mixing with the volumes of the two rivers. Error bars indicate the concentration range measured for duplicate samples.

The distance between stations 9 and station 8 is approximately 3.5 km. The significant linear relationship ($p < 0.05$) obtained between observed and expected total PFAA concentrations indicates that the soluble PFAAs are transported long distances with no apparent loss in fresh water systems (Figure 4.8B). The deviations to the observed conservative behavior are PFOA, which exhibits loss falling below the mixing line, and PFOS which is above the conservative mixing line indicating an additional source between Stations 9 and 8. However, of the PFAAs studied, both PFOS and PFOA were observed to fluctuate in concentrations in the effluent of WWTPs studied along the Housatonic River over a one week period and therefore are more prone to variability in the rate of input.

Conservative mixing was also observed for PFAAs along the lower region of the Housatonic as the river waters meet the LIS estuary waters. Composition profiles were consistent along river surface waters (Figure 4.10). Decreasing PFAA concentrations along the river followed the salinity mixing line, therefore were found to be consistent with conservative mixing behavior (Figure 4.11). PFAAs are shown to continue their conservative mixing behavior along the Housatonic River as it meets the saline estuary waters; the salt intrusion is detectable at station 5 with a salinity of 11.2 ppt. Sampling station 5 is directly downstream of the Milford WWTP. The effluent pipeline discharge zone is located centrally in the river, with the diffusion pipe opening up from the river bed. Thus effluent discharges from Milford are engineered to be well mixed with the river waters. However, the impact of the discharge is apparent in the increased PFC concentrations recorded at sampling station 5 (Figure 4.10).

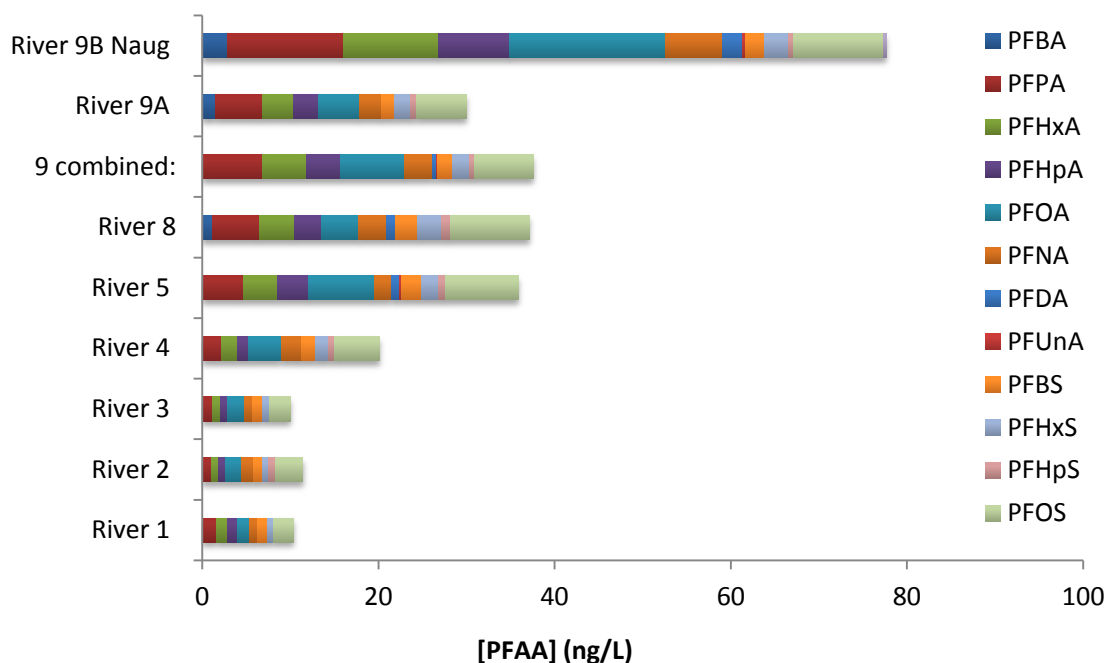


Figure 4.10: Concentrations and composition profiles of PFAAs measured in surface river waters along the Housatonic River, July 2012.

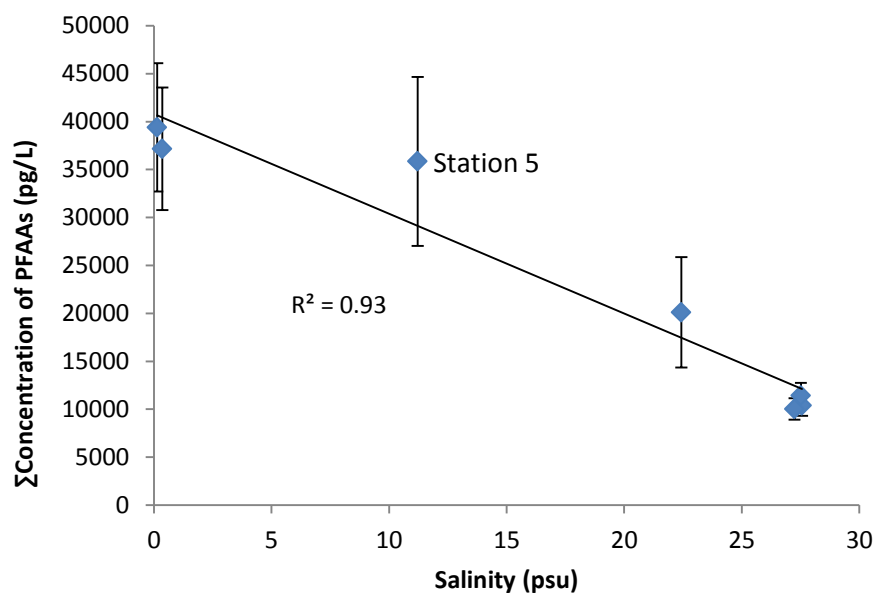


Figure 4.11: Total dissolved PFAA concentrations along the salinity gradient of the Housatonic River as it mixes with the saline estuary waters. Concentrations at station 9 are the sum of the mixing PFAA mass flows at 9A and 9B where the Naugatuck meets the Housatonic River. Error bars indicate the concentration range measured for duplicate samples.

For hydrophobic contaminants, such as PCBs, increasing salinity in natural systems is known to moderately enhance partitioning of contaminants into sediments or suspended sedimentary materials as the presence of salt reduces the effective aqueous solubility (the aqueous activity coefficient). As hydrophobic contaminants are by nature sparingly soluble, increased salinity and turbidity at the brackish water regions where river and estuarine waters mix are known traps for these contaminants, due to increased adherence to and burial in bed sediments as particulate material undergoes resuspension and flocculation in this zone.

Partitioning of PFAAs to natural sediments has been shown to be a function of sediment organic carbon content as well as solution chemistry (2). As sorption to organic carbon (K_{oc}) increases with increasing perfluoro-alkyl chain length (0.5-0.8 log unit per CF_2 moiety; see chapter 3), hydrophobic interactions are suggested to be a dominant mechanism in partitioning. At increased salinities however, electrostatic interactions lead to enhanced sorption of PFAAs to particulate organic matter (POM), due to the presence of common seawater divalent cations, Ca^{2+} or Mg^{2+} , which interact both with the negatively charged surface of POM and the anionic head group of the PFAAs, forming a cation bridge (2, 43). Decreased solution pH has been reported to reduce the partitioning capacities of PFAAs (2) however studies have shown that the presence of divalent cations in solution resulted in the reverse of this effect, where PFAA sorption was found to increase with increasing solution pH (43, 44 and references therein). This phenomenon is suggested to be attributed to the development of more basic sites on the POM surface to which divalent ions can bond with the resulting enhanced PFAA sorption via the cation bridging mechanism.

Jeon *et al.* (45) reported increased partitioning (K_d) for PFOS, PFOA, PFDA and PFUnA, by a factor of 2.1 – 2.7, to particulate matter with increasing water salinities, and Pan and You (13) reported increasing K_d values for PFOS between water and sediments with increasing salinity along the Yangtze River (26). Based on these observations, it was hypothesized that, as with classical hydrophobic organo-halide pollutants, there would be a degree of increased partitioning of PFAA to SPM-POM and/or bed sediments in saline estuarine waters compared to fresh riverine waters in the HR, and trapping of PFAAs in the turbid environs where fresh and salt waters mix. The data obtained in this study concerning the concentrations of PFAAs in surface waters along the HR however do not support the hypothesis of increased partitioning due to increasing salinity. Dissolved PFAA concentrations predicted based on the measured concentrations observed at station 8 were found to account for the concentrations observed in surface waters at station 5 with a high degree of correlation ($R^2 = 0.77$) therefore indicating that mass flux is also conserved into the saline estuarine waters (Figure 4.12).

River water samples were obtained from stations 1, 2, 3 and 4 from on board the R/V Lowell Weicker; samples from both surface and near bottom waters could therefore be obtained for these stations. PFAA concentrations measured in both surface and deep waters at stations 1, 2 and 3 show both consistent compositions profiles and concentrations, thus the waters in this region of the river appear to be well mixed, as is also reflected in the salinity profile (Figure 4.13).

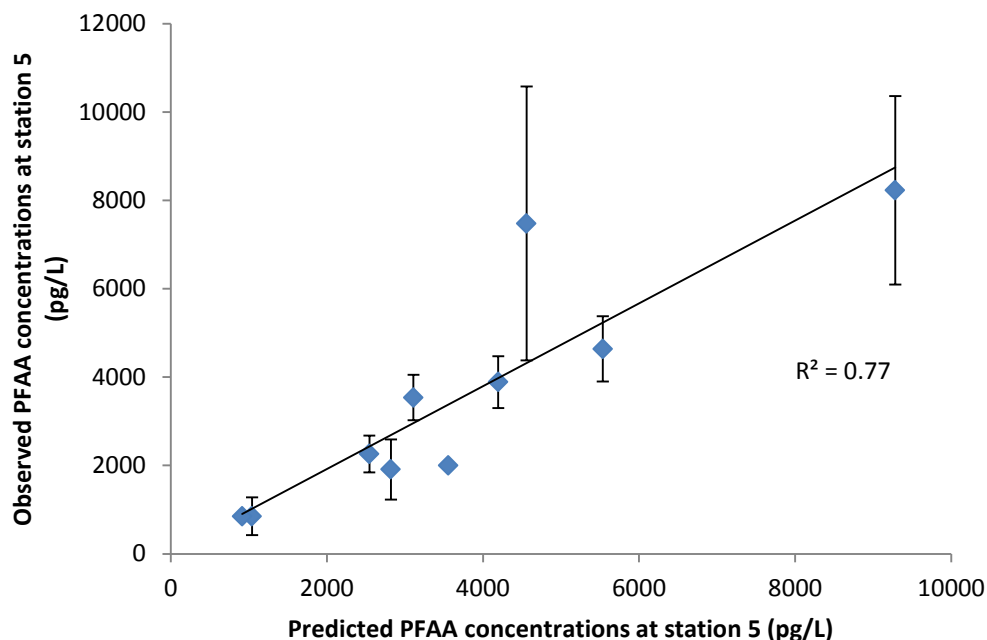


Figure 4.12: Predicated (calculated) PFAA concentrations for station 5 compared to PFAA concentrations measured. Error bars indicate the concentration range measured for duplicate samples.

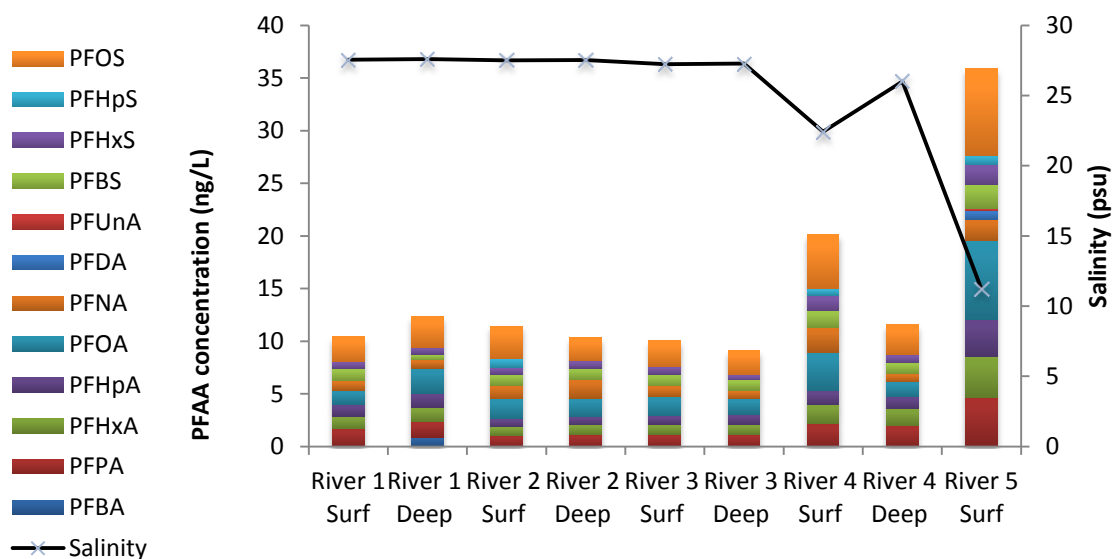


Figure 4.13: Σ Concentrations of PFAAs in the surface and deep waters in the Housatonic River Estuary, July 2012.

The trend of decreasing PFAA concentrations towards the river mouth is consistent with mixing of higher PFAA concentration up-river waters with low PFAA concentration salty estuary waters for every target PFAA in this study; the distributions of each individual PFCA and PFSA along the salinity gradient of the Housatonic River surface waters are given in Figures 4.14 and 4.15 respectively. Each PFAA can be described as undergoing conservative mixing along the salinity line. The longer chained PFCAs, PFOA and PFNA, showed weaker correlations than the more soluble shorter chained PFCAs, PFBA and PFHxA, which would be expected due to increased sorption and potential losses to sediments of the more hydrophobic compounds, however, it is also likely in this case that fluctuation of the longer chained species is due to the greater variability in the inputs of these PFCAs from WWTPs, as observed in the effluent from Beaverbrook WWTP from week 1 to 2.

The input of PFAAs from Milford WWTP was clearly detected at station 5 as indicated by concentrations elevated above the linear trend for a number of PFAAs. Conversely, the linear trend for PFBS, the shorter chained and more soluble PFSA is not as clear as that of the longer chained PFSAs, PFHxS and PFOS, though the variability observed at these very low concentrations is well within the variability observed in UPLC-MS/MS replicate sample analysis (70-130%) and the measured concentration range between duplicate extracted samples.

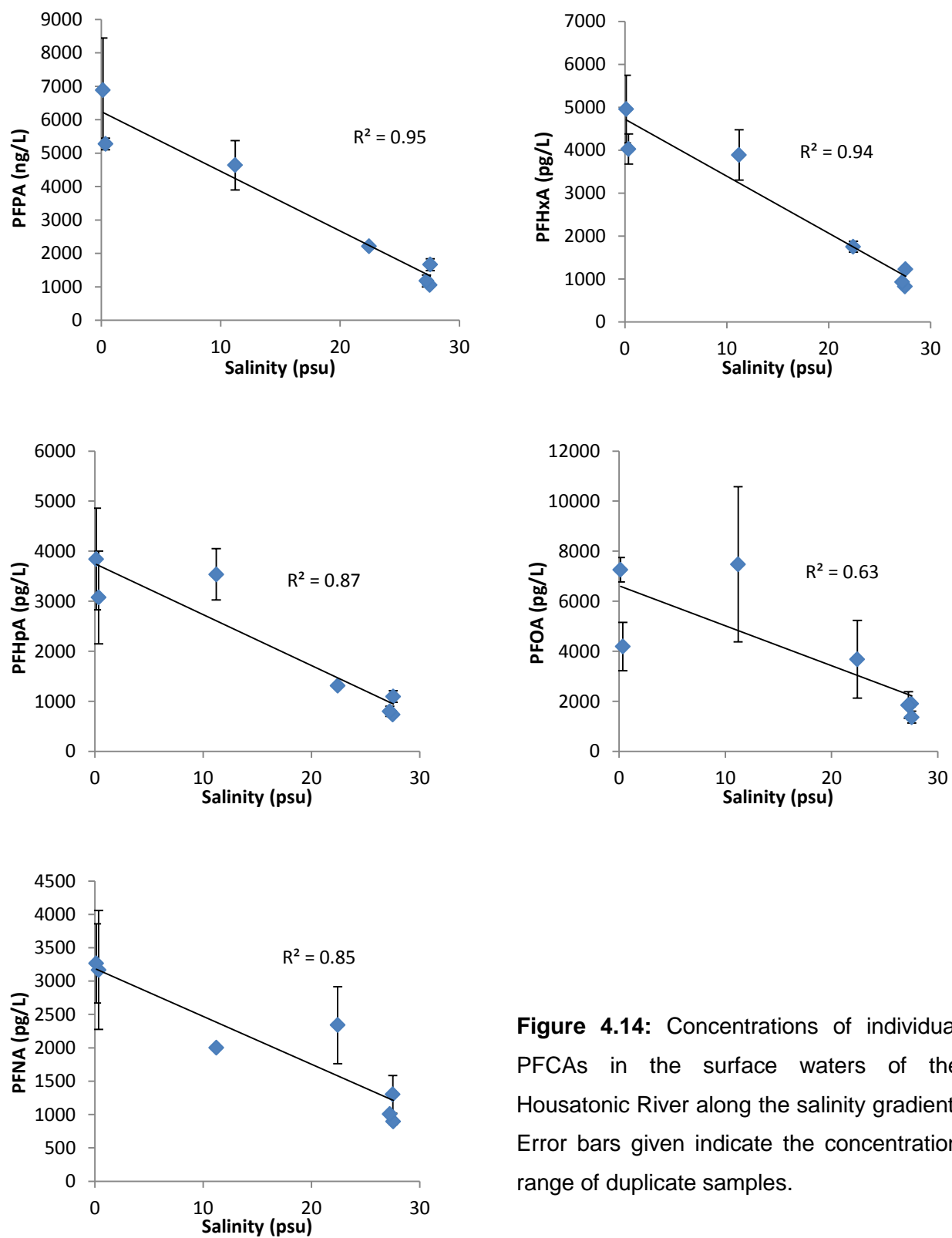


Figure 4.14: Concentrations of individual PFCAs in the surface waters of the Housatonic River along the salinity gradient. Error bars given indicate the concentration range of duplicate samples.

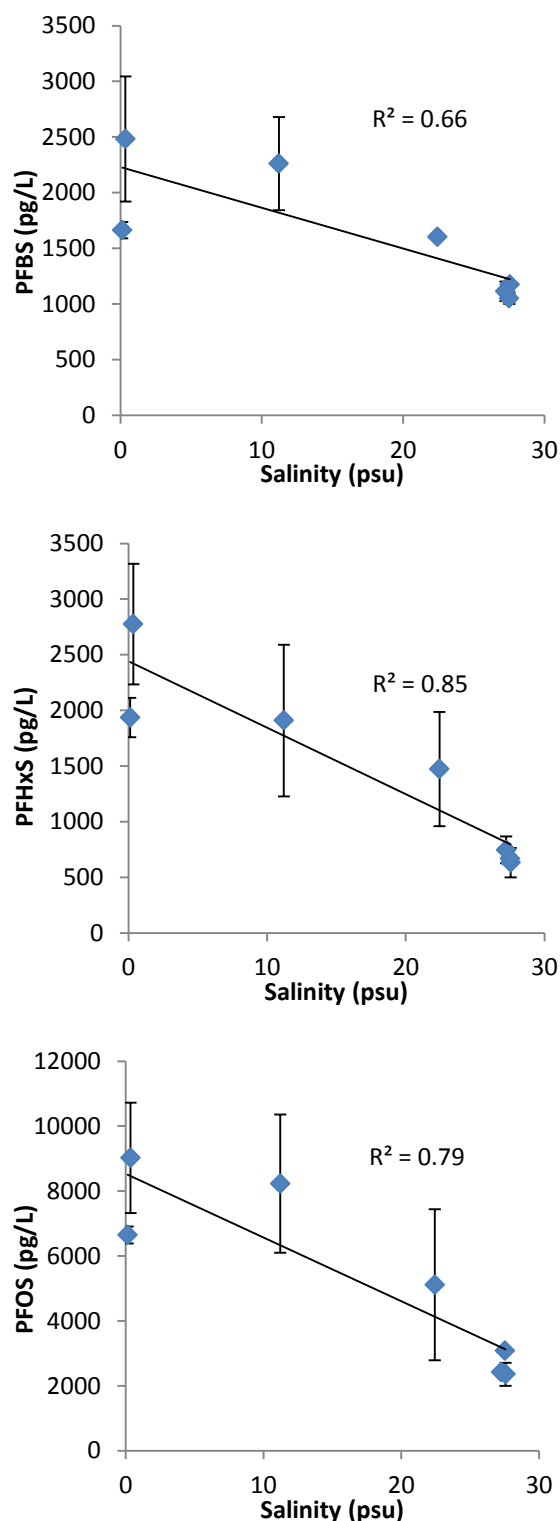


Figure 4.15: Concentrations of individual PFASs in the surface waters of the Housatonic River along a salinity gradient.

Using the concentrations measured at river station 8, due to the known volume flux of the HR at that location, the daily mass flux of total target PFASs down the Housatonic River into the LIS was calculated to be an average (range) of 72 (61 – 87) g/day during the summer low river discharge conditions.

4.4.1.3 PFAS concentrations in LIS

The Housatonic River plume waters in the LIS were sampled on the third day of consecutive field sampling, July 25th, from on board the *R/V Lowell Weicker*. PFAS concentrations were tracked either side of the plume as it moved out into the LIS estuary, in order to assess the potential exposure areas in the Sound. Sampling began at river stations 4 and 1, which had been sampled the previous day. Plume waters were tracked several kilometers off shore and three times further along the coast to the west, beyond the city of

Bridgeport. River transport pathways vary with river discharge rates, tides, wind conditions and other factors. Model simulation results from Michael Whitney (personal communication) show the Housatonic River waters have residence times of over 1 month in the LIS, and that the HR plume pathway tends towards the west, thus can transport contaminants towards the western LIS, an area heavily impacted due to high population density, and ecologically stressed with seasonal hypoxia. In order to track the distribution of PFAAs in the river plume into the LIS, sampling locations were determined from surface salinity measurements, assuming the lower salinities are associated with the HR plume.

Total PFAAs, as well as all individual PFAAs measured, again exhibited a clear linear trend consistent with mixing of higher concentration HR waters with lower concentrations LIS estuarine waters (Figure 4.16). Conservative mixing behavior is also illustrated by each individual PFAA detected in HR plume waters, to varying degrees of significance, as detailed in Figures 4.17 and 4.18. In particular, the longer chained PFCAs, PFOA and PFNA, that were detected in the surface water samples out into the LIS displayed the greatest range and unpredictability in concentrations, which could again be explained by the fluctuations in WWTP input and the variability of instrumental analysis at these low concentrations. However this may reflect the importance of non-point source loading, such as urban run-off, in this shoreline region, based on the observations of Nishikoori *et al.*(46) who reported that unlike PFHxS and PFOS, non-point sources contributed greatly to PFOA and PFNA riverine concentrations (Iruma River, Japan), using boron as a tracer of WWTP point source emissions.

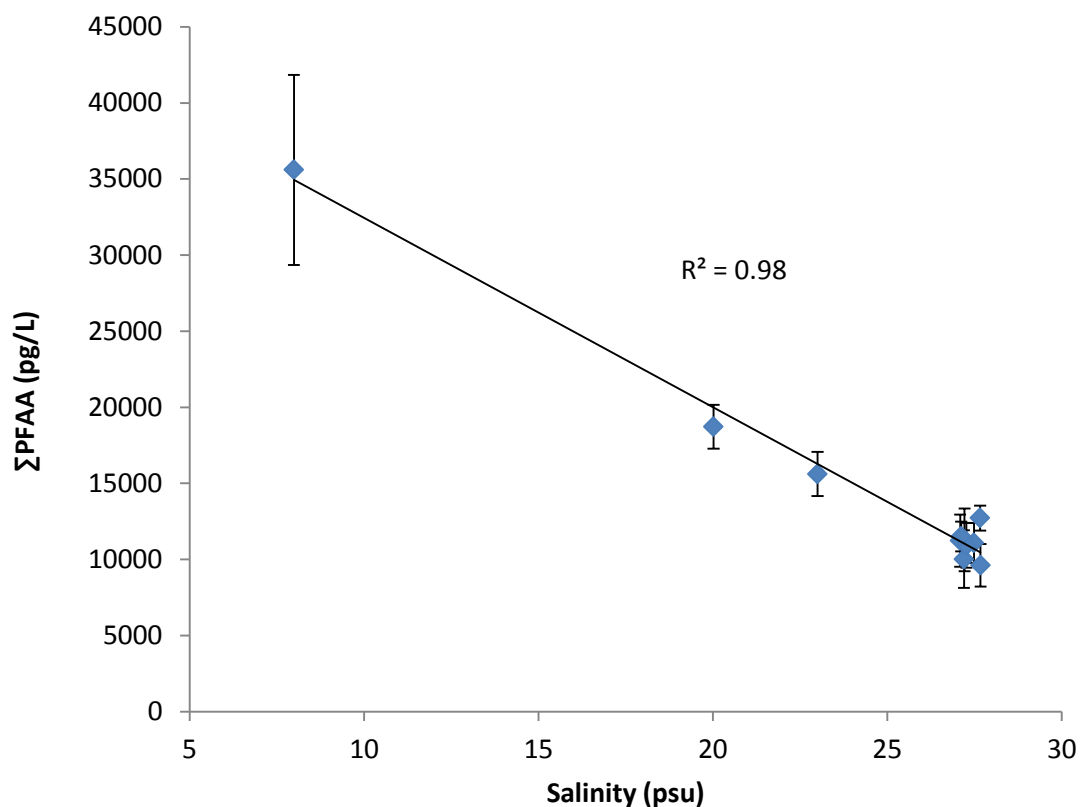


Figure 4.16: ΣConcentrations of PFAAs in the surface water of the Housatonic River plume, July 25th 2012.

The concentrations of PFAAs in the surface waters of the estuary were found to be generally consistent at all sites in the LIS surface waters. PFAAs in the dissolved phase of the river waters do not appear to be lost when the fresh waters enter the saline estuary water. Though the salinity and turbidity of the estuary are hypothesized to be a trap and sink for hydrophobic organic pollutants, it appears that dissolved PFAAs remain so. These linear trends are likely to be also reflective of the behavior of PFAAs as they move from the LIS out into the open ocean.

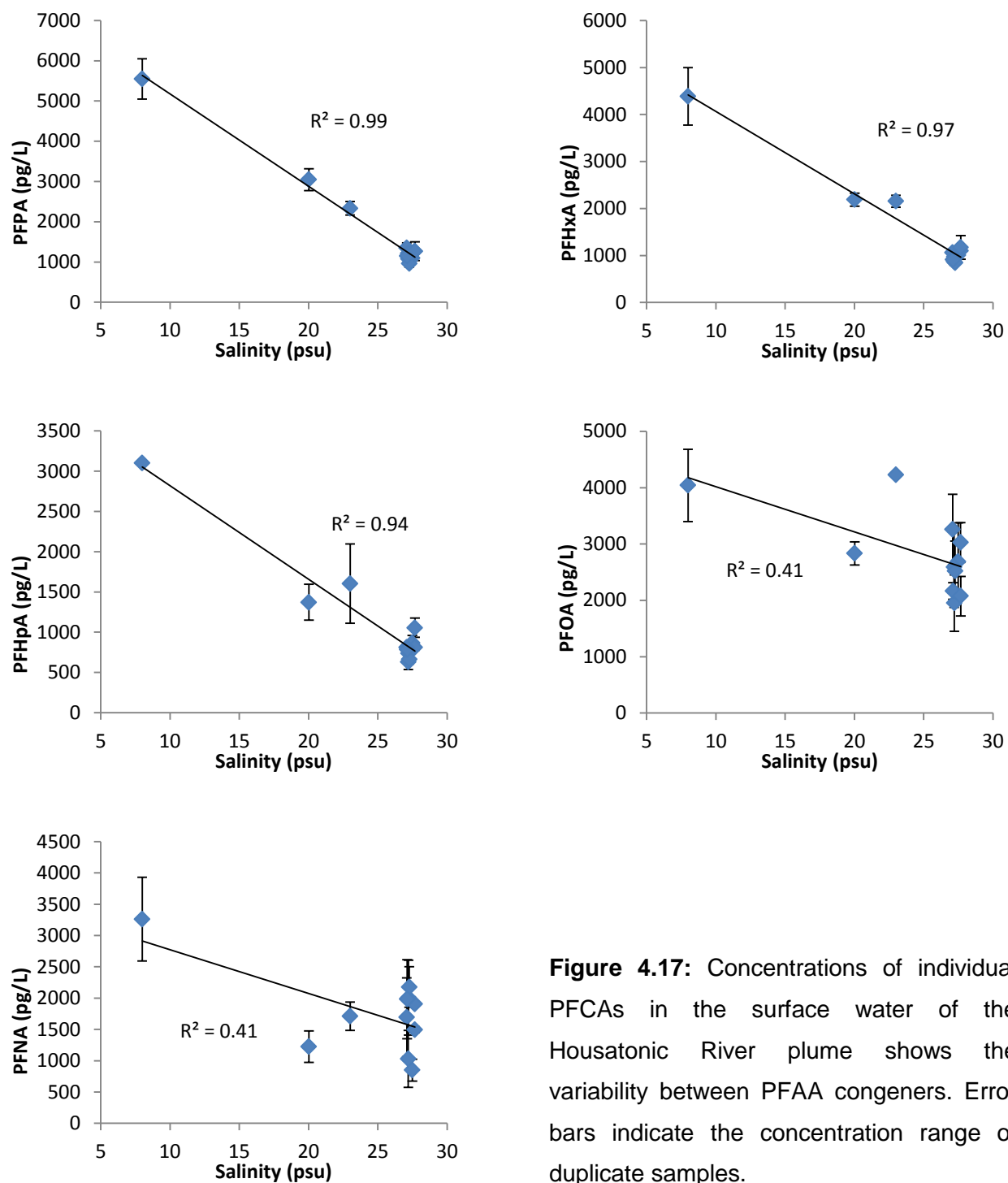


Figure 4.17: Concentrations of individual PFCAs in the surface water of the Housatonic River plume shows the variability between PFAA congeners. Error bars indicate the concentration range of duplicate samples.

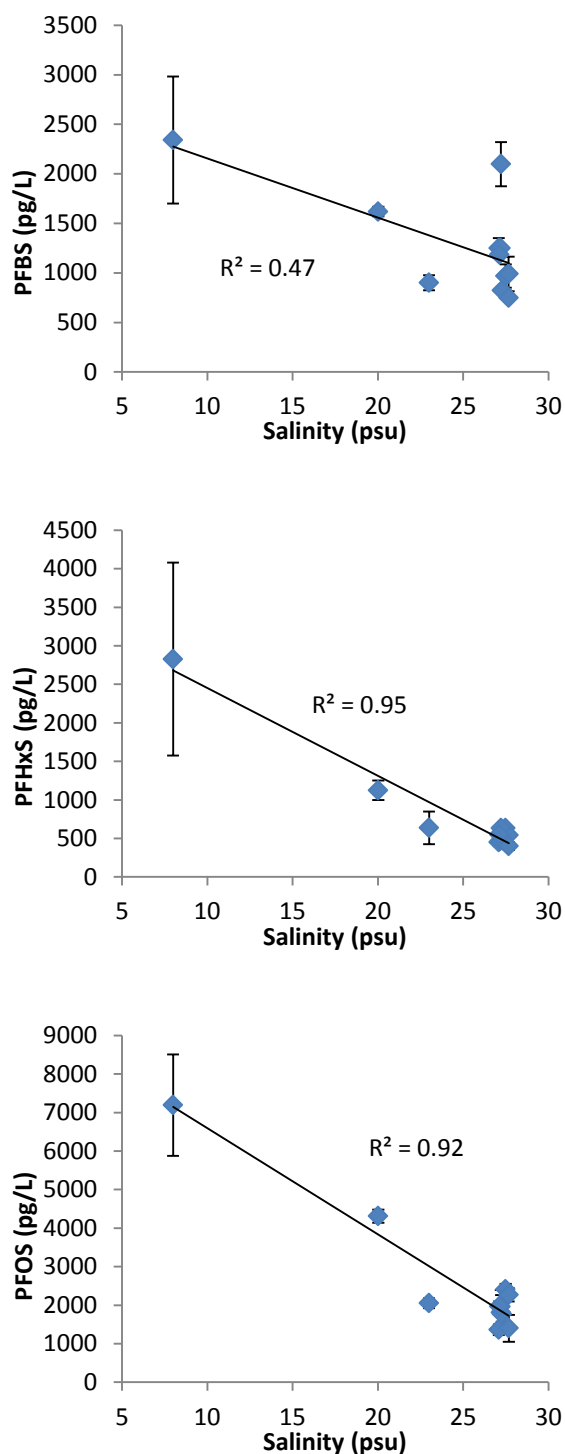


Figure 4.18: Concentrations of individual PFSA's measured in the surface water of the Housatonic River plume.

The effluent discharge zone for the Stratford WWTP was located 1020 m upstream of the HR sampling station 2. No surface water samples, from the HR or LIS, showed any particular increases in concentrations downstream of the effluent discharge zone, unlike the PFAA measured at HR station 5, where concentrations of several PFAAs detected were elevated above the salinity mixing line, indicative of Milford WWTP input. The effluent outfall from Stratford WWTP is a stream that runs out into the west side of the HR and into a salt marsh. It is highly likely therefore that the marsh area acts as a trap for PFAAs. The peak in PFOA observed at LIS sampling station 4P, located just west of Stratford along the coastline, may be due to Stratford discharge, however PFPA, the PFAA measured in the greatest concentration in Stratford WWTP final effluent and a more soluble

PFAA, has no increase in concentration in HR or LIS waters, but displays dilution into the LIS following the salinity mixing line in a linear fashion. Run-off from shore in the proximity of LIS station 4P may have contributed to the elevated PFOA concentrations at this location, possibly originating from Sikorski Airport in this region. However, as the salinity at LIS 4P is lower than the other LIS stations (23 ppt compared to 27 ppt), the elevated PFOA seen at LIS 4P is most likely coming from the HR plume.

4.4.2 Spring high flow

A second field survey was conducted during June 2013 in the Housatonic River and estuary during high riverine discharge conditions. The river flow was 10 times higher than during the summer low-flow survey, with a flow rate of 220 m³/s recorded by the USGS gage located at Stevenson Dam. Due to a 50% budget reduction in research funding, the observational program was modified from that of the previous year. The mass flux of PFAAs along the Housatonic River was estimated using two sampling sites- river station 8 and river station 1. In order to gauge the input from WWTPs, two of the previous six WWTPs were sampled- Milford and Stratford, both located near the river mouth and with the largest effluent volume discharge rates of the WWTPs located in the lower Housatonic, have the biggest impact on PFAA mass loadings to the HR plume waters. Upriver station 8, plus the final effluents of Milford and Stratford were sampled both one week prior and one day prior to HR and LIS water sampling. For the day 2 modified observational program, water samples were collected from near-surface and near-bottom waters for river station 1, at the Housatonic mouth, and from one LIS plume station (LIS 20) close to a commercial shellfish area near the mouth, throughout a complete tidal cycle.

4.4.2.1 PFAA concentrations in wastewater effluent

Average daily flow rates for the two WWTPs sampled prior to the spring high-flow survey showed slight but consistent increases with respect to the prior year summer survey, with an increase factor of 1.1 (4.8 MGD to 5.5 MGD) for Milford and 1.2 (5.5 to 5.8 MGD) for the Stratford WWTP. Total measured concentrations of PFAAs in the final effluent (dissolved plus particulate phases) are given in Table 4.6. Compared to the prior year lower flow survey, only the concentration of 1 out of the 7 PFCAs detected showed any major change; PFBA 3x lower in spring at both WWTPs, with concentrations decreasing from around 11 to 3 ng/L. Concentrations of PFPA, PFHxA, PFHpA and PFOA were the same during spring and summer. However, PFNA and PFDA were only detected in effluent in the spring. Overall the average Σ PFAA concentrations at the Milford WWTP (average of both weeks) marginally decreased from 133.5 ng/L to 111.1 ng/L during spring high flow, a decrease factor of 1.2, mirroring the increase in flow rate to the WWTP. This observation highlights a consistency in the rate and source of inputs of either PFCAs or PFCA precursor compounds to the Milford WWTP. Average total PFAA mass flux from Milford WWTP was calculated as 2.44 g/day summer low-flow and 2.64 g/day spring high-flow.

PFCA concentrations measured at the Stratford WWTP showed no significant change between spring and summer; with total PFCA concentrations of 89.9 ng/L for summer low flow and 85.7 in spring high-flow. However, concentrations of PFSA were found to be from 3x to an estimated 1000x higher in the spring survey. Concentrations of all 4 target PFSA increased, with values for the first week (one week prior to the spring HR survey) notably greater (2-5x approximated) than the PFSA concentrations

measured the following week (Figure 4.19 and Table 4.6). The consistency in the higher PFSA concentrations measured over the course of the two WWTP samplings validate the legitimacy of the data, and point to a specific pollutant release event as being the cause. Total PFCA mass flux from Stratford WWTP was consistent between the two sampling seasons however with the addition of the PFSA pollution event, total PFAA mass flows increased from 2.31 - 2.97 g/day to 83.5 g/day (May 30th 2013) and 38 g/day (June 4th 2013).

The aircraft manufacturing plant Sikorski (a division of United Technologies Company) is located in Trumbull, just north of Stratford, on the Housatonic River, and discharges waste to the Stratford WWTP. Discharge monitoring reports for 2012 and 2013 obtained from the CT DEEP showed an increase in both average monthly flow and maximum daily flow data for the month of June in 2013, with respect the flow data for July 2012, as shown in Figure 4.20. As airports and industrial wastes are both known sources of PFAA to the aquatic environment (22) it is possible this is the source of the PFSAAs to the Stratford WWTP.

Table 4.6: Σ PFAA (dissolved plus particulate fractions) concentrations [average \pm (range)] observed in final effluent samples from the two WWTPs located near the mouth of the Housatonic River; spring 2013. For values with no given range, n=1 (particulate fraction only). PFOS* values measured in Stratford effluent were greater than the highest calibration standard therefore should be regarded as approximate concentrations of high magnitude.

PFAA	Milford WWTP		Stratford WWTP	
	May 30	June 4 th	May 30	June 4 th
PFBA		3.4 (0.2)	2.4 (0.4)	3.1 (0.0)
PFPA	32.5 (2.2)	22.8 (0.1)	30.0 (1.5)	24.3 (1.3)
PFHxA	12.4 (0.5)	9 (1.4)	16.4 (1.7)	10.1 (1.0)
PFHpA	5.0 (0.5)	2.9 (1.8)	5.6 (1.7)	3.8 (0.8)
PFOA	45.2 (18.2)	46.7 (20.9)	24.1 (1.3)	19.5 (5.2)
PFNA	2.5 (1.3)	5.1 (1.4)		0.6 (0.0)
PFDA	3.2	7.8 (0.1)	18.0 (0.0)	13.5 (1.2)
PFBS			32.7 (7.7)	6.9 (1.7)
PFHxS	4.3 (3.2)	0.5 (0.1)	72.8 (1.7)	16.1 (0.8)
PFOS	19.5 (2.3)	33.4 (15.4)	2849* (130)	1198* (226)
PFDS			24.7 (0.4)	16.2 (1.1)

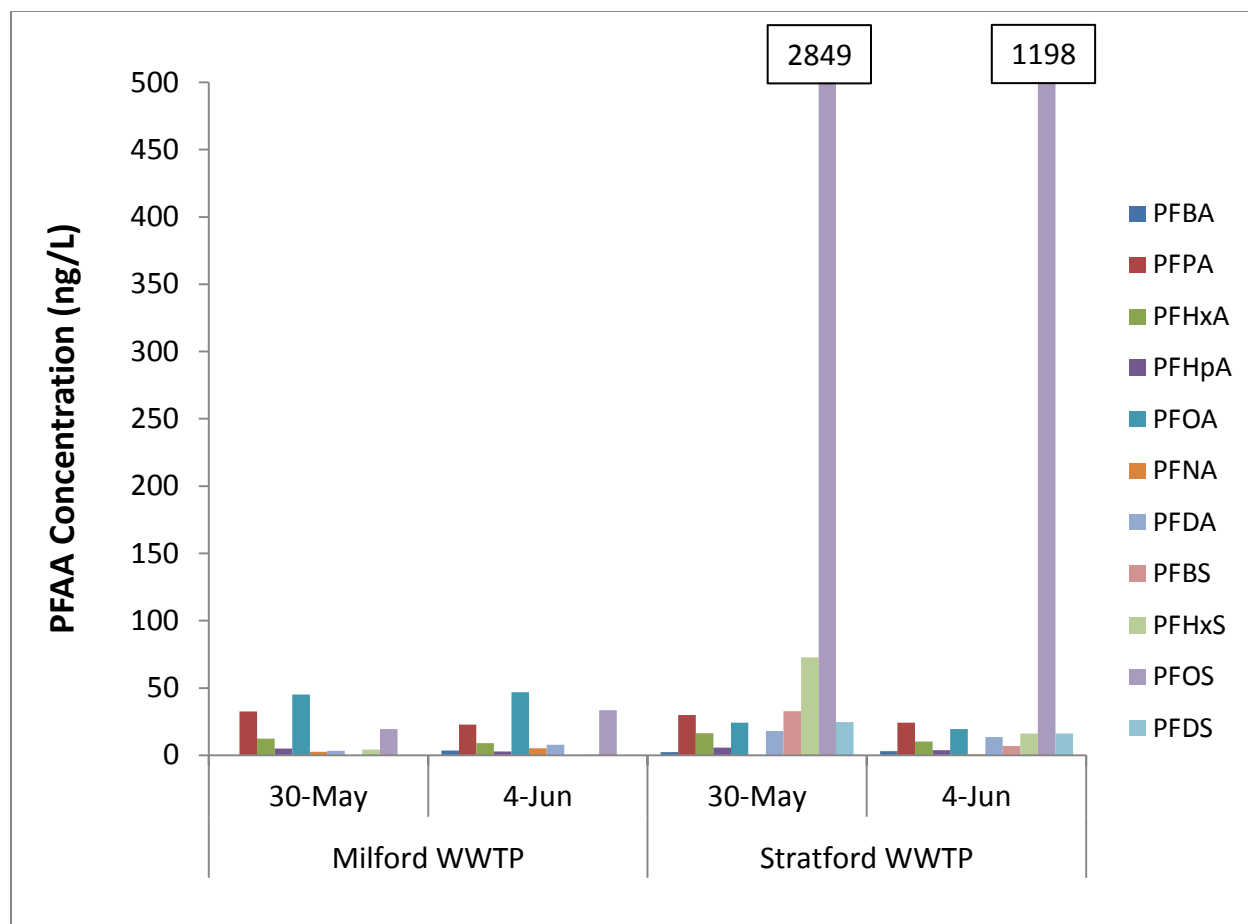


Figure 4.19: Σ PFAA (dissolved plus particulate fractions) concentrations observed in final effluent samples from the two WWTPs located near the mouth of the Housatonic River; spring 2013.

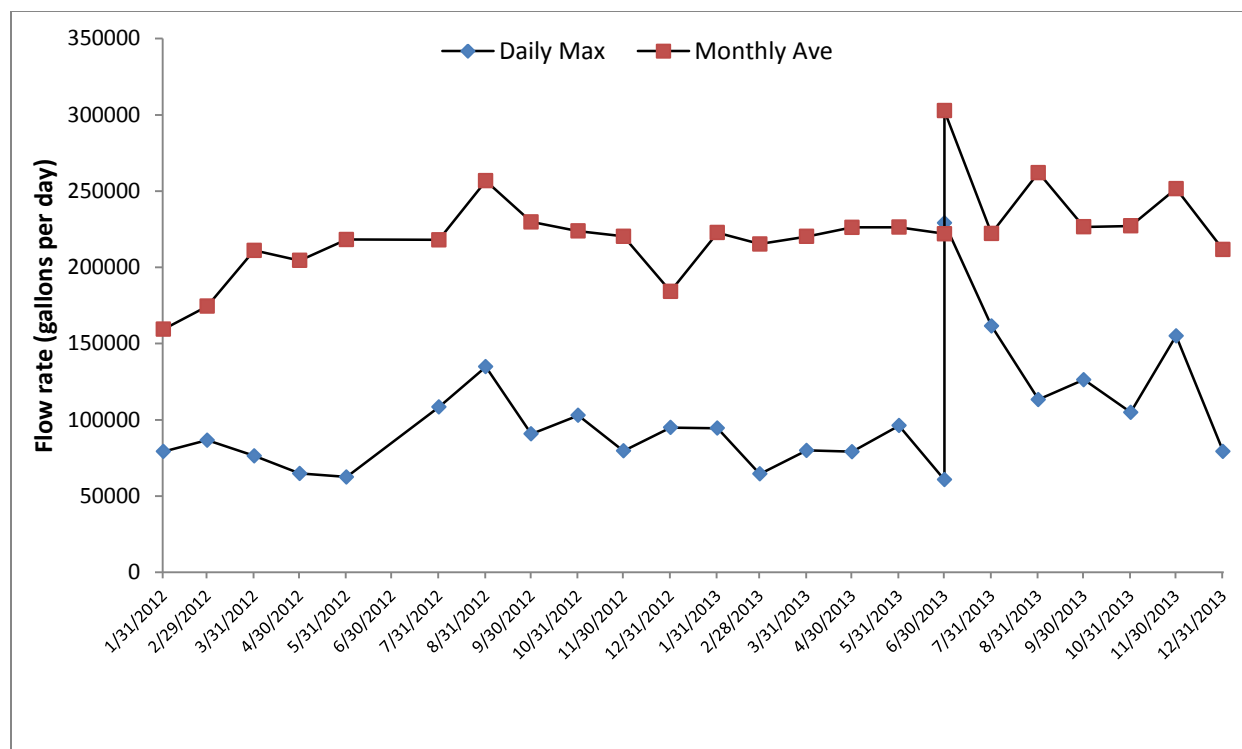


Figure 4.20: Flow rate data from Sikorsky Aircraft; discharge permit CTP000551 annual summary report.

PFAA concentrations measured in effluent, riverine and estuarine waters during the summer low-flow and spring high-flow survey had, to this point, been detected at concentrations far below the predicted no-effect concentration values for protecting wildlife of 25 µg/L in freshwater and 2.5 µg/L in marine waters (47). However the concentrations of PFOS discharging into the lower Housatonic estuary from the Stratford WWTP from the discharge event in June 2013 may be, during these pollutant release events, within the range of the marine water no-effect concentration value, within the proximity of the effluent discharge zone, particularly since the tertiary disinfection process in the Stratford WWTP is UV, therefore the effluent discharge flow

is continuous. The salt march located outside the effluent discharge zone for the Stratford WWTP is very likely a trap for effluent derived contaminants, accumulation of PFOS and PFDS in this region may be of concern to wildlife in this area, particularly given the tendency of these longer chains PFSA's to bioaccumulate and biomagnify in the food chain (48).

4.4.2.2 Influence of hydrological regime of PFAA levels in surface waters

PFAA concentrations measured in the Houston River surface waters were lower during spring high river discharge than during low river flow hydrology as would be predicted due to dilution in a greater volume of water. The decrease in average Σ PFAA concentrations measured at HR station 8 was from 36 ng/L (low flow) to 13 ng/L (high flow), and from 14 ng/L to 4 ng/L, high to low flow at HR station 1. A total of 6 PFAAs were detected in the river at stations 8 and 1, with PFPA, PFOA and PFOS present in the highest concentrations (Table 4.7), consistent with that of the low hydrology conditions. PFAAs detected were in the dissolved phase only; no PFAAs were found associated with the particulate phase. The negative correlation between concentrations of PFAAs and river flow signifies the importance of point sources as origins of PFAAs to the river system, which become diluted when river flow increases. In addition, the concentration ratios between PFOA and PFOS, and between PFPA and PFHpA, observed in the river waters are in good agreement with those observed in WWTP effluent in both high and low flow regimes (Figure 4.21) with the exception of the large PFSA polluting event from the Stratford WWTP, spring 2013, which was not included in this assessment. Similar correlations were also found for PFPA/PFHpA for the WWTPs, river stations 1 and 8

sampled in both spring high flow ($R^2 = 0.93$, $p < 0.0005$) and summer low flow ($R^2 = 0.80$, $p < 0.01$) regimes, and for PFHxA/PFHpA during high ($R^2 = 0.96$, $p < 0.0005$) and low flow ($R^2 = 0.80$, $p < 0.01$). Labadie *et al.* (28) found similar agreement with PFOA and PFOS in the River Seine, France, as did Möller *et al.* (49) for the River Rhine, suggesting similar, likely domestic, waste sources and dynamics in those river systems. The strong concentration ratio correlations for the PFAAs detected in both WWTP effluent and river waters on both spring and summer surveys suggests a similar source of these PFAAs under both hydrological regimes. Thus WWTPs are the most likely sources of PFAAs to the HR and LIS aquatic system under high and low river discharge conditions.

The ratio of PFOA/PFOS observed in Milford and Stratford effluent and river waters (stations 1 and 8) was found to have a linear correlation with flow rate under summer low-flow ($R^2 = 0.52$, $p = 0.057$), however no correlation was observed in the spring in the same locations (Stratford omitted) spring high flow, which indicates that although there are noteworthy correlations between the concentration ratios of the PFAAs observed in effluent and river stations 1 and 8 in low and high flow conditions, the contributions of diffuse, non-point sources during high river discharge cannot be excluded, particularly considering the small number of data points made in these comparisons due to reduced sampling in the revised spring survey.

Table 4.7: Concentrations (ng/L) of PFAAs detected in river surface waters, during high river discharge condition, spring 2013.

Sampling Station	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFOS
River 8 - 05/30/2013	1.4	1.9	1.0	1.4	5.7	3.1
River 8 - 06/04/2013	0.9	2.7	0.9	0.6	4.4	2.4
River 1 (average) 06/05/2013	0.2	0.5	0.6	0.3	1.6	1.0

The average mass flux of total PFAAs in the Housatonic surface waters increased an estimated 3x in the spring compared to the summer, from 71.7 g/day to 249 g/day at river station 8, and 28.7 g/day to 77.7 g/day in the mixed estuarine waters at the river mouth (station 1). The mass flux of PFOA increased 10x greater in spring high flow at both river sampling stations, increasing from 8.3 g/day to 95.7 g/day at HR station 8 and from 3.5 g/day to 30.2 g/day at station 1, in direct relation to the 10x increase in river discharge volume. Total PFAA and PFOA mass fluxes at the WWTPs and river stations 1 and 8 are compared in spring and summer in Figure 4.22.

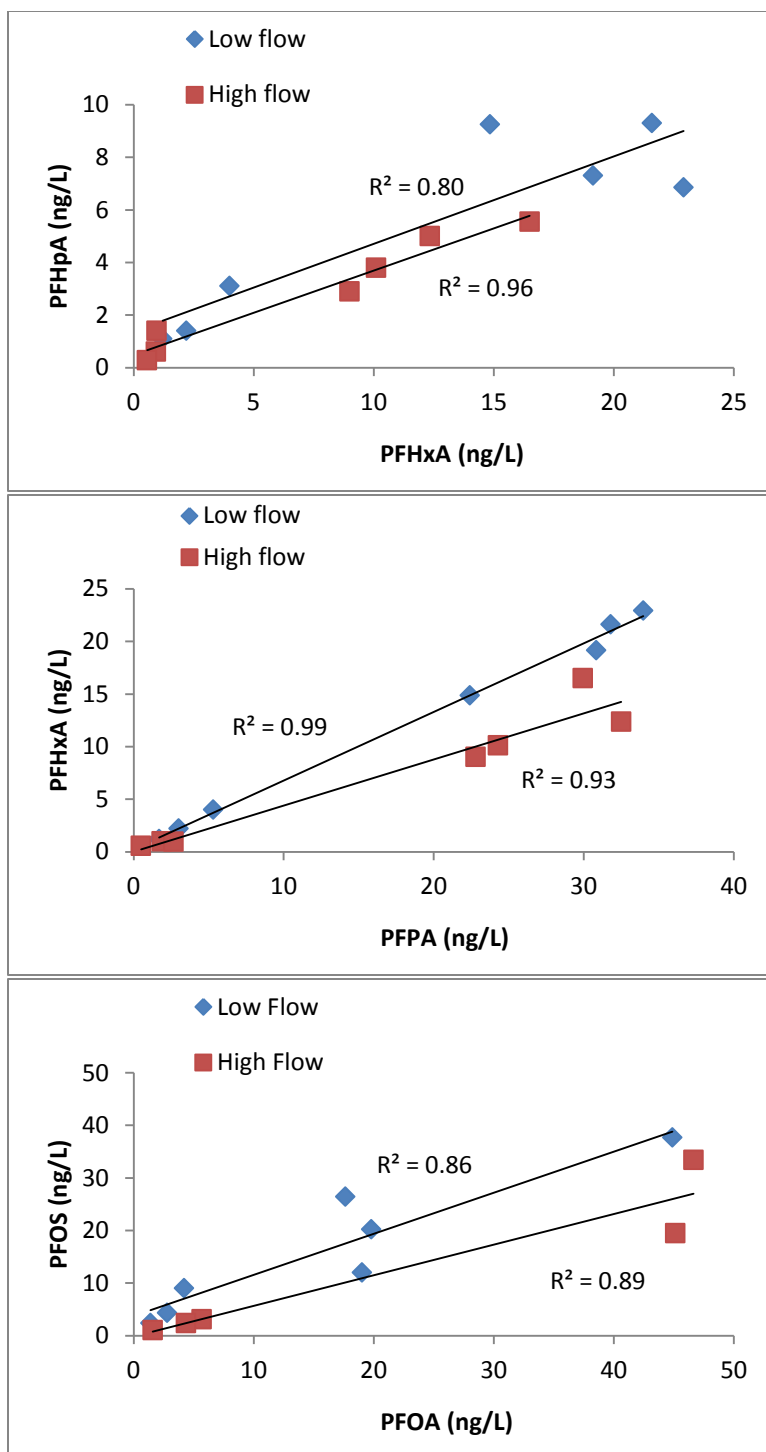


Figure 4.21: Correlations between PFPA and PFHxA (top); PFHxA and PFPA (middle), and PFOA and PFOS (bottom) in the 2 WWTPs and in surface waters of river station 1 and 8 surveyed summer low flow and spring high-flow. The PFOS concentrations in effluent from Stratford WWTP in spring 2013 were excluded from the spring high flow calculations.

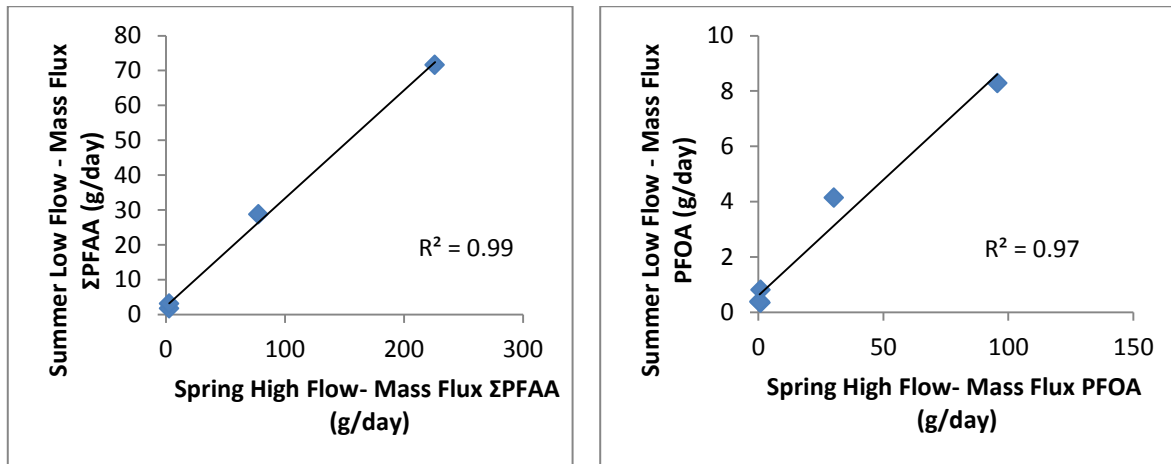


Figure 4.22: Mass flux of (left)- Σ PFAAs (sum of all congeners) ($p=0.001$) and (right)- PFOA ($p<0.0005$) is strongly correlated with flow rate. Three point groups represent the two WWTPs and river sampling stations 1 (river mouth) and 8 (up river). The mass flux from the Stratford WWTP was excluded in the comparisons of the Σ PFAA congener mass flux (left) due to the anomalously large efflux of PFSAAs in the spring of 2013.

4.4.3 PFAAs in the upper Housatonic River

A third, small scale survey of water and sediments was conducted during low river discharge conditions, October 2014, in order to estimate the mass loadings of PFAAs originating from the upper Housatonic. River surface water was collected from two locations above the Derby effluent discharge zone, as shown in Figure 4.23; the upper Derby site was located 650 meters up river of the WWTP pipeline, and the upper river site was located above the Derby-Shelton Dam, 4.5 km above the effluent discharge zone. River surface water samples were collected at HR stations 9A, 9B and 5.

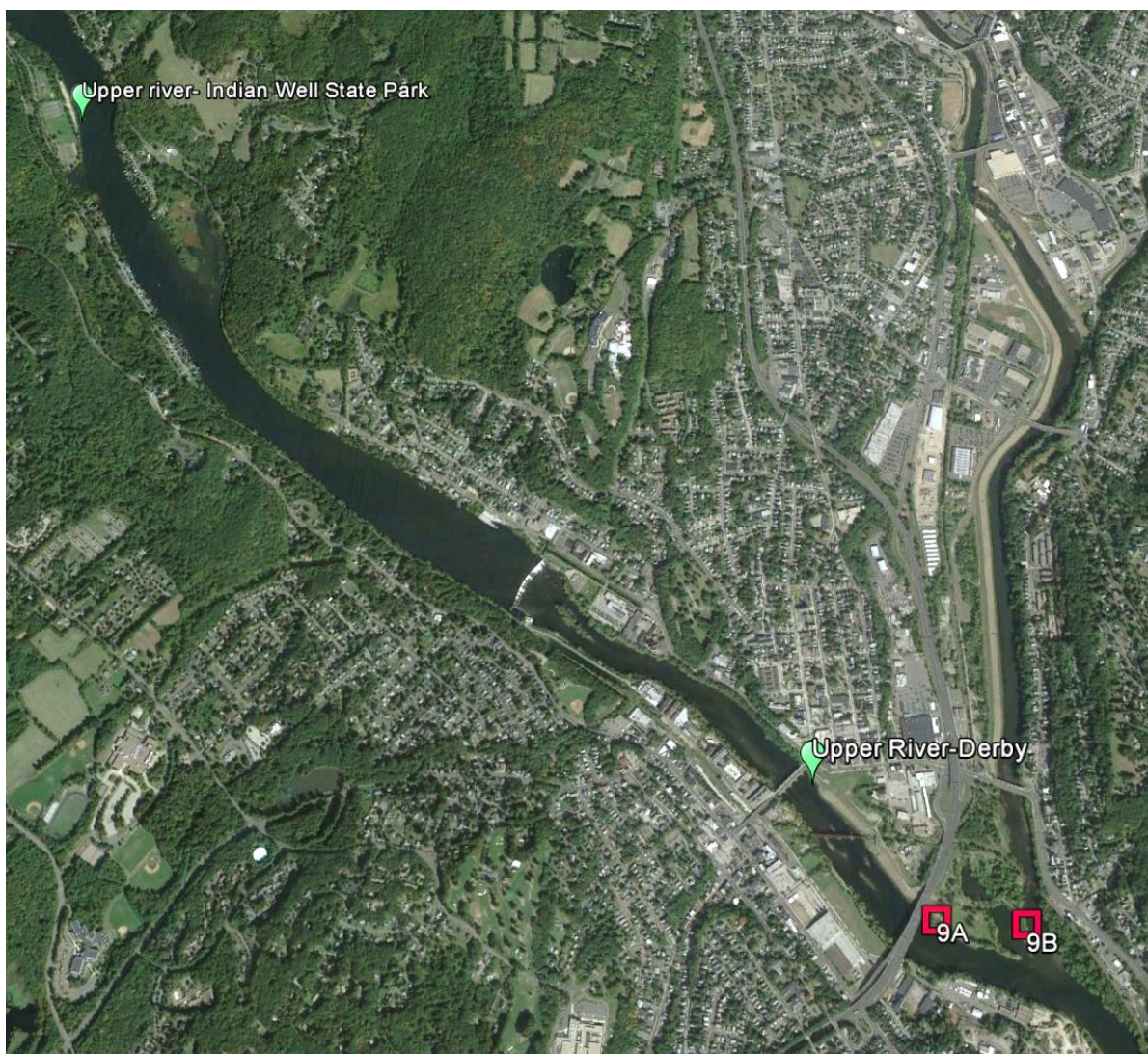


Figure 4.23: Locations of up-river sites sampled on October 2014, under a low river discharge hydrology.

Results from the river surface water samples collected October 2014 were consistent in both concentrations and composition profiles of PFAAs detected at HR sampling stations 9A, 9B and 5 during the July 2012 (Figure 4.24). PFAAs measured in the 2 sites on the upper Housatonic were also consistent with the PFAAs measured at HR station 9A. These results indicate that the PFAAs measured in HR 9A July 2012

were a reflection of the large up-river source of PFAAs to the Housatonic, and not a reflection of a direct sampling of the Derby effluent plume, as had been previously considered. Based on this conclusion, and the comparison of the mass fluxes from the Housatonic and Naugatuck Rivers, Shelton WWTP, and the concentrations measured at River station 8, 80% of the proportion of PFAA loadings to the Housatonic River was estimated to be coming from source(s) further up river. Additionally, given the consistency of the concentrations detected in Indian well State park, upper Derby and lower Derby sampling sites (survey 3, October 2014), the sources of the PFAAs are likely to be north of Indian Well State Park. Potential sources include the several WWTPs located along the Housatonic and major tributaries north of Indian Well, as previously discussed in section 4.4.1.2

The consistency of the concentration and composition profiles strongly suggested the origin of PFAAs to the upper Housatonic during low-river discharge hydrology to be primarily point-sources, most likely WWTP effluent, with a stable PFAA signature indicative of WWTPs with predominantly domestic waste. Comparison of the PFAA data obtained at 9B (Naugatuck River) and HR station 5 found the same distinct agreement in PFAAs detected, which again strongly supports the hypothesis of WWTP loading being the source of PFAAs to the river, with PFAA profiles at HR station 5 being more variable than those seen at 9A and 9B, likely due to the proximity to Milford WWTP discharge, which is released continuously. Ascertaining the stability of the PFAA values measured at these locations in both July 2012 and October 2014 is highly valuable, as this allows for further assessment of the impact of WWTP derived PFAA

loadings to the HR and by extension to the other major LIS tributaries in this region, with a high degree of confidence.

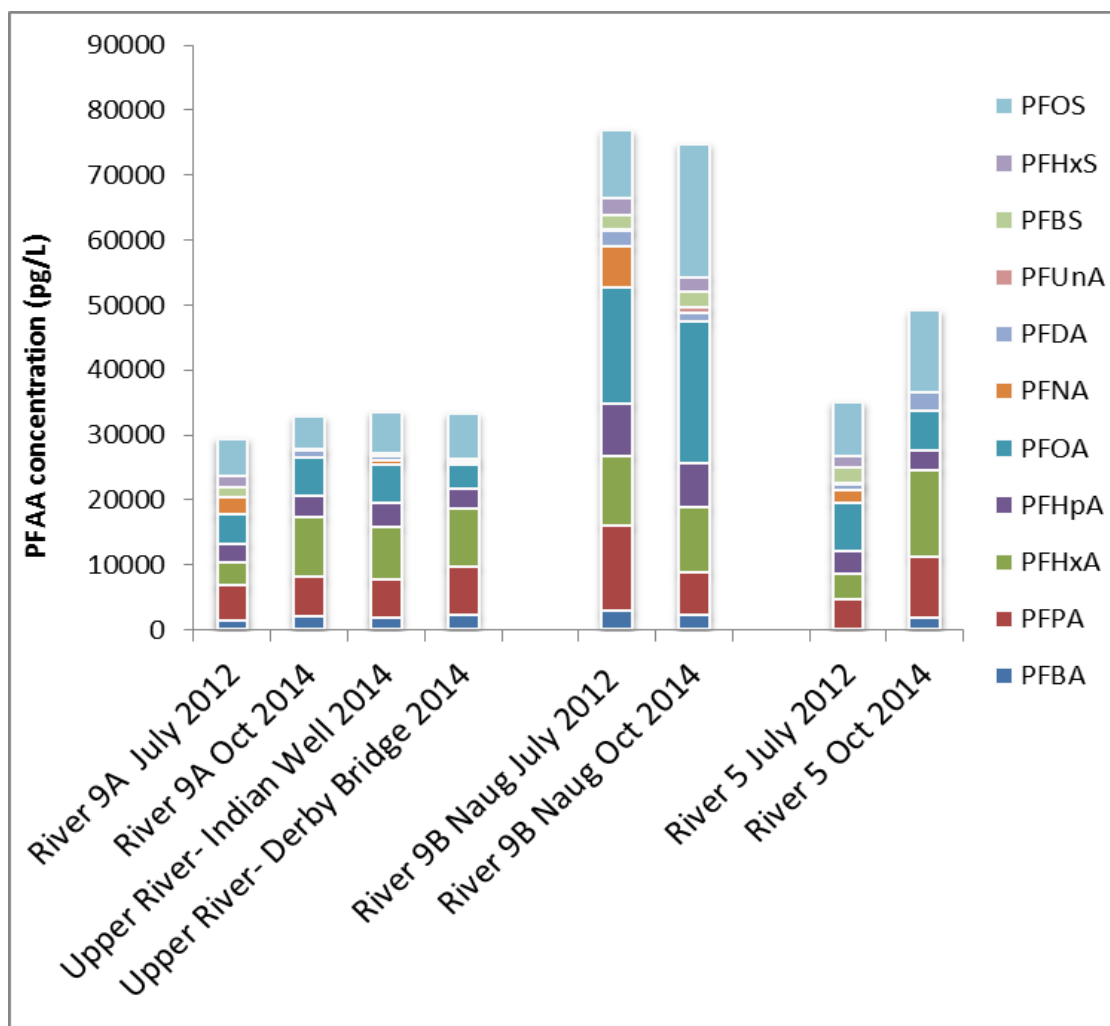


Figure 4.24: Concentrations and composition profiles of PFAAs in samples collected October 14th 2014 from stations 5, 9A and 9B plus two upper Housatonic locations above the Derby WWTP effluent discharge site, compared to PFAAs detected in the same locations, July 2012.

4.4.4 Particle-water partitioning of PFAAs

Partitioning coefficients measured in field studies are useful as they describe a range of relationships which are not often seen in laboratory batch experiments, due to the heterogeneity of the aquatic environments, differing chemical and physical characteristics of particulate organic matter, including presence of micro-organisms for example, that cannot be controlled. An important fraction of the organic materials suspended in freshwater systems consist of the recalcitrant remains of woody terrestrial plants, whereas the organic materials suspended in the waters of estuaries are typically derived from terrestrial and aquatic organisms remains (50). Sorption of organic pollutants to particulate organic matter (POM) may involve partitioning into (absorption) as well as on to (adsorption) the variety of different organic phases that can be present. Therefore field based calculations of K_{oc} values can be as variable as the chemistry of the POM between different riverine and estuarine systems. It is important to obtain field based observations in many different aquatic systems in order to complement data obtained in controlled laboratory experiments, to provide insights on variability and ranges in values of physical parameters, which are important for modeling the transport and fate of pollutants.

The SPM collected from on board the *R/V Lowell Weicker*, in the river surface waters along the salinity gradient of the Housatonic, July 2012, was analyzed for carbon, nitrogen, and for $\delta^{13}\text{C}$ stable isotopes. The carbon isotope profile obtained for SPM collected along the salinity gradient also confirmed the $\delta^{13}\text{C}$ signal in the fresh up-river waters to be of terrestrial origin (Figure 4.25), with the $\delta^{13}\text{C}$ of the POC in the riverine end member being more depleted than that of the marine end member waters.

This trend is typical of estuarine systems, such as that observed in the Delaware estuary, where the $\delta^{13}\text{C}$ signal in the riverine end member (~ -24 to -31) was more depleted than the marine end-member (~ -22 to -24), and is attributed to up-river input of terrestrial derived organic matter (51 and references therein).

PFOS was the only PFAA detected in the SPM phase of both river and estuary water samples during the low river discharge survey July 2012. Higher particulate PFOS concentrations were seen in samples from the up-river stations, particularly at 9B- Naugatuck River and at stations 8 and 5 on July 24th, and at river station 4 on July 5th. Concentrations of particulate bound PFOS, in ng per liter of water, were low overall, ranging from 0.1 – 1.5 ng/L; duplicate samples of 2 liters of water from each station were filtered in order to collect enough SPM to detect any PFAAs in this phase. Particulate fraction PFOS concentrations correlated with the isotopic $\delta^{13}\text{C}$ signal in the SPM along the river, with higher PFOS concentrations found associated in the upriver POM, and lower SPM-PFOS concentrations with the estuarine SPM with $\delta^{13}\text{C}$ values around -22 to -23 (Figure 4.26). Higher PFOS concentrations in the SPM phase would be expected in the upper river water samples, since the concentrations of PFOS concurrently measured in the aqueous phase were also higher.

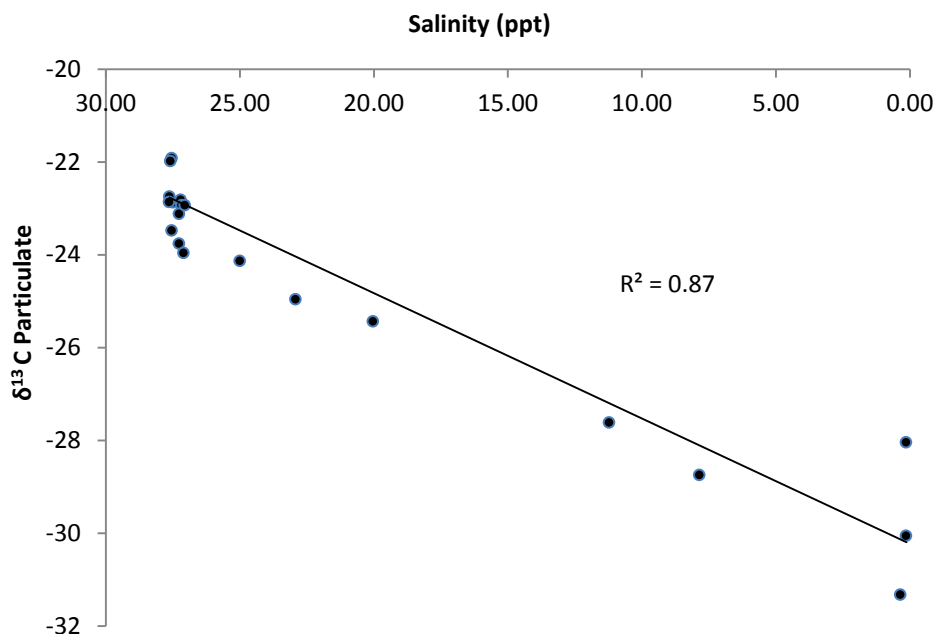


Figure 4.25: $\delta^{13}\text{C}$ profile of particulate organic matter in surface waters along the Housatonic River and estuary during summer low-flow conditions, July 24th and 25th.

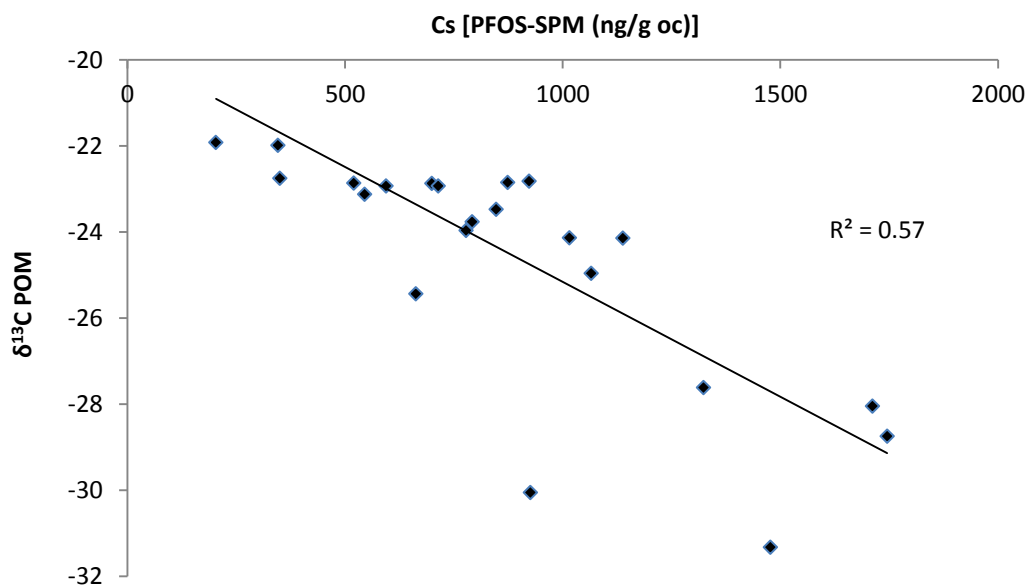


Figure 4.26: $\delta^{13}\text{C}$ profile of particulate organic matter (POM) in surface waters along the Housatonic River and estuary compared to the concentration of PFOS associated with the SPM fraction.

Sorption isotherms describing the relationship between the concentration of PFOS in the surface water solution and in the suspended particulate matter phases were determined for the field observations on July 24th and July 25th (Figure 4.27). Although data from both survey days does show a positive linear relationship between solid and solute PFOS concentrations (Figure 4.27-A), a more significant linear relationship was observed in the upper river water samples (Figure 4.27-B). The equilibrium distribution of PFOS in the upper river is best described by a linear isotherm (Figure 4.27-B) indicating that the partitioning of PFOS is governed by the hydrophobic interaction of the perfluoroalkyl chain, partitioning into a homogenous organic phase where the strongest adsorption sites are far from being saturated (49).

While the data did not fit the Freundlich (Figure 4.27-C) and Langmuir (Figure 4.27-D) quantitative isotherm models as well as the linear model, the Freundlich model was a better fit out of the two. Previous laboratory based studies have however determined that the Langmuir model usually describes the sorption isotherms of PFOS and PFOA well (43 and references therein), however the Freundlich isotherm has also been successfully applied with coefficients ranging from 0.75 to 1.00 (2). These previous studies however were investigating the partitioning of bed sediments, or settleable solids. The Freundlich exponent ($n_i = 0.52$) derived for the PFOS aqueous-SPM July 24th data from the linear regression equation (Figure 4.27-C), which is in the form of:

$$\log C_{is} = n_i \log C_{iw} + \log K_{iF}$$

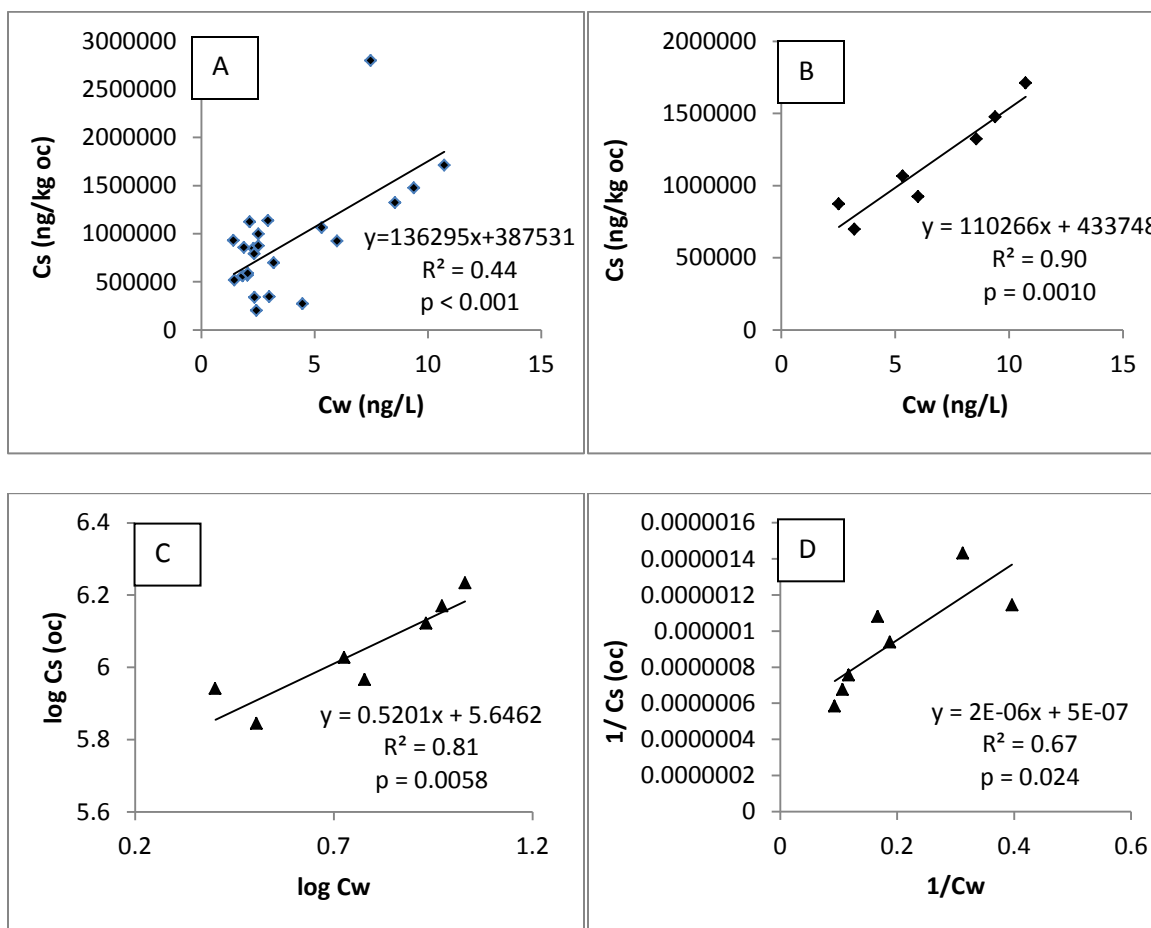


Figure 4.27: The distribution of PFOS between the suspended solids and solution present in (A) the Housatonic River and LIS surface waters July 24th and 25th, and (B) between SPM and water in HR surface waters, from river stations 2 to 9, July 24th only. Distribution patterns describing sorption of PFOS between suspended particles and water, July 24th HR stations 2-9 only; (C) Freundlich and (D) Langmuir isotherms.

The non-linearity of the Freundlich isotherm contradicts the linearity of the sorption relationship implied by Figure 4.27-B. In this case, the field data set obtained for the up-river Housatonic samples, $n_i < 1$, therefore it may be interpreted that the amount of sorption is smaller in proportion to the amount of PFOS in solution. In this field data however, salinity must also be addressed, as the higher PFOS concentrations

in both dissolved and particulate phases is greater in the upper fresh water regions. The Freundlich exponent of <1 obtained therefore could also infer that the sorbate, PFOS, is bound with weaker free energies in the upper fresh river water compared with in the saline estuary waters, indicating that the aqueous activity coefficient ($\gamma_{\text{PFOS,w}}$) increases with salt concentration. This describes a 'salting-out' behavior of PFOS, which is not consistent with the conservative mixing behaviors described earlier for the PFAA concentrations measured along the surface waters of the Housatonic River and into the LIS.

The effect of salinity was further investigated by comparing the partitioning coefficients derived for samples obtained at each station along the river. Log K_{oc} values derived for all stations, where PFOS was detected in both dissolved and SPM phases ranged from 4.7 – 5.8. Although the previously determined filter artifact value was applied, the partitioning constants derived are higher than previously published values. Derived log K_{oc} values, while showing variability, were not observed to fluctuate as a function of salinity (Figure 4.28). The lack of relationship between salinity and partitioning constants may be attributed to the limited number of observations in this study. Jeon *et al.* (45) found, in a lab-based study, that for PFOS, PFOA, PFDA and PFUnA, the distribution coefficient (k_d) describing partitioning between water and particles increased by 2.1 - 2.7 fold with increasing water salinity from 10 – 34 ppt, and estimated a salting constant in the range of 0.80 - 1.11 (45). In a different study, Xiao *et al.* (52) reported the detection of PFOS in the particulate fraction in samples of storm-water run-off from the Twin Cities (Minneapolis and St. Paul, MN), in concentration levels so high that the solid-water partitioning constants derived were orders of

magnitude greater than those previously published. The authors concluded with the possibility of PFOS containing particles entering the wastewater stream, from sources that could include debris of textiles and carpet and several industrial polymers. The SPM phase PFOS measured along the Housatonic River may consist of PFOS containing particulates that do not actively undergo any partitioning processes, and the linear relationship depicted in Figure 4.27-B is a reflection of the dilution of PFOS in both phases along the river. This hypothesis would be consistent with the high partitioning constants derived and the observation of no salinity effect on partitioning.

In order to investigate the field observations, a laboratory experiment was conducted using water samples obtained from riverine and estuarine end members; at the Upper Housatonic River site (Indian Well State Park), and from Stratford Marina. Water samples were mixed in ratios to produce samples with salinities of 0, 1, 2, 5, 15 and 26 ppt. Duplicate samples were spiked with native PFAA mixtures and rolled overnight prior to filtration. Aqueous and filter/SPM phases were then extracted as previously described. Results from this experiment are displayed in Figure 4.28. PFOA and PFNA were not detected in the SPM phase at concentrations higher than the filter artifact, which is consistent with these PFAAs not detected in the SPM phase in the HR water samples even though aqueous phase concentrations of these PFCAs were comparable to PFOS concentrations in the upper river stations. The data however indicates that salinity did increase partitioning of PFDA, PFUnA, PFOS and PFDS to a small extent (0.5 – 0.8 log units) in the 1 - 2ppt salinity range, with no further enhanced partitioning with increasing salinity (Figure 4.29). PFAAs are dissociated in ambient water; the presence of the cations with the increase of salinity likely results in the

formation of strong ion pairs, increasing the hydrophobic interaction between the POM and the anion by effectively neutralizing the negative charged moieties on both PFAA and on the surface of the POM.

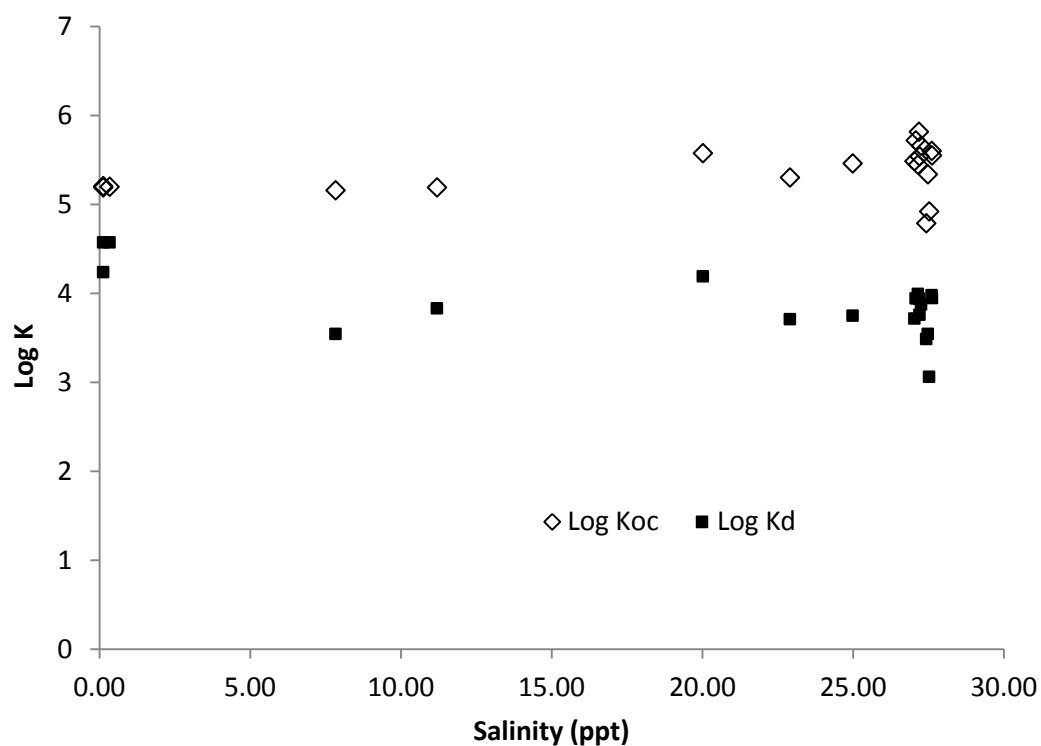


Figure 4.28: Field derived Log K_d and K_{oc} values for PFOS measured in water and suspended particulate phases, July 2012 (low flow conditions).

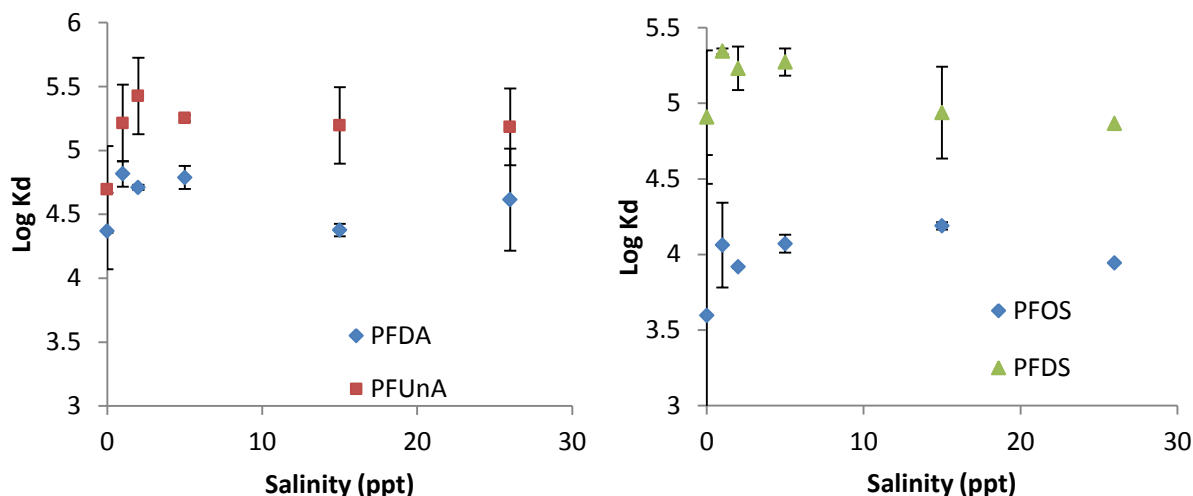


Figure 4.29: Lab-based investigations into salinity effect on water-SPM partitioning using samples of river and estuary waters mixed in different ratios and spiked with native PFAA compounds. Error bars given are range (n=2).

Increasing sorption passed the 2 ppt salinity range was not observed, contrary to the results of Jeon *et al.* (45), however, it is possible that in this case the field water samples used had limited POM unlike the *Chlorella* cultures used by Jeon *et al.*, therefore sorption sites may have been effectively saturated within the initial salinity range. PFAA concentrations used in this study (ng/L) were far below PFAA solubility or micelle formation concentrations, therefore a ‘salting-out’ effect would not be likely, and initial enhanced partitioning to POM attributed to reduced electrostatic repulsion

The results from the laboratory experiment are also consistent with partitioning being function of perfluoro-alkyl chain length, with longer chain PFAAs displaying greater partitioning to the solid phase, as has been previously reported with laboratory investigations utilizing bed sediments (2, 24, 25, 43). Here, an increase in average K_d value of 0.55 log units was seen with an additional perfluorocarbon moiety for the

PFCAs, and an increase of 0.59 log units for PFSAAs, consistent with the results of Higgins and Luthy (2). Partitioning coefficient values derived were also determined to be consistent with those observed in the field in this study, with the exception of the K_d value observed in fresh water (0 ppt). K_d values obtained for PFOS over the salinity range increased from 3.60 at 0ppt, to 4.06 at 1ppt. The average K_d for the entire salinity range was 3.96 ± 0.29 . The average K_d value obtained for PFOS in the field samples was 3.83 ± 0.36 . These results are remarkably consistent, which lends support to the hypothesis that the presence of PFOS with the SPM fraction of the field samples from the Housatonic River estuary is a function of active partitioning, as opposed to the PFOS containing particulate hypothesis.

PFAA-particulate partitioning was further investigated during the second and third HR field surveys, June 2013 and October 2014. No PFAAs were detected in the SPM phase in the spring high-river discharge survey. As the PFAA water concentrations were markedly lower, the SPM bound PFAAs may have been too low to detect. PFOA and PFOS were however detected with the SPM phase in samples obtained in October 2014 low-flow conditions; PFOA was detected in the SPM phase in the sampling locations directly downstream from the Milford and Derby WWTP effluent discharge zone. PFOS was detected in the SPM of the samples obtained from the mouth of the Naugatuck River (Station 9B). The PFAAs detected and partitioning constants derived, following correction for filter artifact, are given in Table 4.7. PFOA was only detected in the SPM directly downstream of the two WWTP discharge zones, yet was detected in the dissolved phase at the up river site (Indian Well state park) in the same concentration (5.7 ng/L) as the down-river Derby site (6.1 ng/L), as well as in the

Naugatuck River (9B) at higher aqueous concentrations (21.7 ng/L), equivalent to the concentrations measured in the dissolved Naugatuck water (21.4 ng/L).

The observation of equivalent aqueous phase PFOA and PFOS concentrations, but only PFOS detected in the SPM phase may illustrate the greater tendency of PFOS to partition to SPM compared to PFOA, Ahrens *et al.* (53) reported SPM derived log K_{oc} values for PFOS was 1.3 log units greater than for PFOA. However this could also be a reflection of the presence of PFOS containing particles that are capable of being transported long distances whereas PFOA partitioned SPM is not transported as far from the proximity of the emission source. The detection of PFOS in the SPM of the Naugatuck river but not in the Milford or Derby sites may also be a function of the higher aqueous phase concentration of PFOS in the Naugatuck (21.5 ng/L) compared to Milford (12.5 ng/L) and Derby (5.0 ng/L) as the elemental analysis of SPM from Milford, Derby and from the Naugatuck River found that the organic carbon and nitrogen compositions of SPM at all locations were remarkably similar (Table 4.8).

The log K_{oc} value, 4.9, derived for PFOS in the SPM phase in the Naugatuck River was consistent with the range of SPM derived partitioning constants obtained in survey 1 (4.8 - 5.8); more specifically comparable to the log K_{oc} value in the upper river station 9B (5.2). Field-based SPM- K_{oc} values derived for PFOA (4.4 ± 0.1) and PFOS (5.1 ± 0.5) (were 1.6 – 2 orders of magnitude greater than literature values of 2.8 for PFOA and 3.0 for PFOS, previously published for batch sediment partitioning experiments by Higgins and Luthy (2). The SPM derived log K_{oc} value for PFOS in this survey is consistent with that derived by Ahrens *et al.* (25) of 4.8 ± 0.1 for PFOS on SPM in Tokyo Bay. Ahrens *et al.* also reported an SPM derived log K_{oc} for PFOA of 3.5

± 0.1 , which is lower than the SPM derived coefficient from PFOA of 4.4 in this study. The average PFOA SPM derived $\log K_{oc}$ for survey 3 (4.4 ± 0.1) and the average PFOS $\log K_{oc}$ (5.1 ± 0.2) for the Housatonic freshwater samples from surveys 1 and 3, are remarkably consistent to the effluent derived SPM partitioning coefficient $\log K_{oc}$ of 4.7 ± 0.2 for PFOA and 5.2 ± 0.5 for PFOS (Chapter 3 section 3.3.2).

Table 4.8: PFAAs detected in aqueous and SPM phases, survey 3 samples (October 2014), and partitioning constants derived (n=1)

PFAA	Sample site	PFAA-aqueous (ng/L)	PFAA SPM (ng/g)	SPM -organic carbon ($\mu\text{g/mL}$) -C/N ratio	$\log K_D$ (L/kg)	$\log K_{oc}$ (L/kg-oc)
PFOA	Derby WWTP	6.1	0.1	0.6	3.2	4.5
	(10m downstream)			10.4		
PFOA	Milford WWTP	6.2	0.2	1.7	2.8	4.3
	(10m downstream)			9.4		
PFOS	Naugatuck (9B)	21.5	1.6	1.0 10.8	3.9	4.9

The results discussed in this section strongly suggest that PFAAs show a greater tendency to partition to SPM than to bed sediments; therefore SPM plays an important role in the fate and transport of PFAAs in the aquatic environment. Field derived partitioning constants are vital for pollutant transport and risk assessment modeling; the application of lab-derived constants may lead to a serious underestimation of the SPM bound fraction of longer-chained PFAAs.

4.4.5 Concentrations of PFAAs in bed sediments

The occurrence and partitioning of PFAAs in the sediments along the lower Housatonic River estuary were investigated during the low and high river discharge regimes in July 2012 and June 2013. No PFAAs were detected in the surface bed sediments of the Housatonic River collected from locations along the central axis of the river at sampling stations 2, 3 and 4. Elemental analysis found that the % carbon composition of these sediments to be low and decreasing towards the river mouth, from 1.2 mg organic carbon (OC)/g sediments dry weight (d.w.) at station 4, to 3.9 mg OC/g sediment d.w. at 2. Station 1 at the mouth of the Housatonic had the lowest organic carbon content (0.7 mg OC/g). An additional sediment grab sample was obtained from the eastern bank of the Housatonic at the same location as the river sampling station 3, but closer to the marsh. The organic carbon content of sediment was reported by Higgins and Luthy (2) to be the dominant parameter affecting PFAA sorption; this mechanism was also reflected in the data obtained in this study. Sediments from the marsh area at station 3 were relatively much higher in organic carbon (21.7 mg OC/g) and correspondingly, more PFAAs were detected in this location (8 out of 16 target PFAAs), and total PFAA concentrations the highest (3098 pg/g) compared to sediments at HR stations 1 (460 pg/g), and LIS stations 3 (3P) (1723 pg/g) and 4 (4P) (278 pg/g) (Figure 4.29). Conversely, PFHxS was detected (460 pg/g) in the surface sediments station 1 where the sediment organic carbon content was the lowest of all samples obtained (0.7 mg OC/g), however the detection of PFHxS at Station 1 was not confirmed by duplicate analysis, whereas sediment PFAAs measured at all other locations were duplicated.

PFOS was the predominant PFAA detected in sediments, similar to the SPM fraction, occurring in 3 out of the 7 sites sampled, and occurring in the highest concentration of all PFAAs detected at River station 3, and LIS station 3. PFOS was the only PFAA detected at LIS 4 (Figure 4.30). Interestingly, sediment PFAA concentrations at LIS 3 were higher than those at 4, even though the net transport of the HR plume has been suggested, using model simulations, to move westward (Michael Whitney, personal communication). This may be reflective of an input source from further up along the coast, such as West Haven/New Haven WWTPs or Quinnipiac River system. However, the organic carbon content of the sediments at LIS 3 were 7x higher than that at LIS 4 (16.2 mg OC/g dry weight compared to 2.2 mg OC/g dw), therefore PFAA concentration variability may be a function of the sediment composition.

PFAAs were also detected in sediment samples obtained from the LIS for the spring high river discharge survey. The two stations sampled either side of the HR plume were located at the river mouth (HR 1/LIS 1P at the same location as survey 1) and station 20, due east of the HR plume region, midway between station 1 and the LIS 3 (Figure 4.30). PFAAs were detected in the sediments in station 20 only, which was also consistent with the higher organic carbon (OC) content of the sediment, 19.6 mg OC/g d.w. compared to 5.3 mg OC/g d.w. at station 1. PFOS was again the predominant PFAA in the sediment; PFDA and PFUnA were also detected. PFOS average concentration was 1150 pg/g at station 20, twice the concentrations measured in the sediments at LIS 3P during the summer low-discharge survey (510 pg/g). PFDA and PFUnA were measured at 800 and 730 pg/g respectively for the spring high-discharge, compared to 330 pg/g and <LOD for the summer high river discharge. This

may be have been a reflection of a decreasing concentration profile away from the Housatonic River mouth PFAA source as LIS 3P was located further east that station 20.

Sediments in the upper area of the Housatonic survey study were sampled in October 2014 under low river discharge conditions. Surface sediments were collected from near shore locations at the upper river-Derby station, and from HR stations 9A (downstream of the Derby WWTP outfall), 9B (Naugatuck River mouth) and 5 (downstream of the Milford WWTP outfall) (Figure 4.23). Even though water phase PFAA concentrations at the two sites either side of the Derby WWTP outfall were found to be consistent, and the water phase PFAA concentrations in the Naugatuck (9B), which did not vary between surveys, were consistently higher than water phase PFAA concentrations at all 3 Housatonic River sites, (Figure 4.24), no PFAAs were detected in the surface sediment samples obtained from the upper river-Derby site, nor from the Naugatuck River mouth station 9B. PFAAs were only detected in the surface sediments directly downstream of the 2 WWTP outfalls, at river stations 9A and 5. Concentrations of PFAAs detected in the sediments at 9A and 5 are given in Table 4.9. PFOS was the predominant PFAA in sediments outside the effluent outfalls, consistent with being the predominant PFAA detected in effluent SPM. These results are consistent with those of Becker *et al.* (42), who reported PFOA and PFOS in concentrations 3x and 40x correspondingly in the sediments compared to water concentrations, downstream of a WWTP discharge zone in Germany. Results in this study show far greater sediment/water ratios, in the order of 130x for PFOA and 300-1000x for PFOS (Table 4.9) however the total organic carbon (TOC) content of the sediment in this study was

also much higher, at 2.7% for Derby outfall and 4.3% for Milford outfall sediment, 1 - 2 orders of magnitude greater than the TOC in the study to Becket *et al.*, and explain why the PFAA concentrations in this study are proportionally greater. Sediment/water ratios increased with increasing perfluoro-alkyl chain length, however no consistent increase was observed with the increased salinity at Milford compared to the fresh water system near Derby WWTP.

Elemental analysis of the sediments obtained from the upper Housatonic River locations confirmed that the organic carbon content of the sediments in the direct downstream proximity of the effluent discharge zone was much greater than in the sediments of the upper Housatonic Site upstream of the Derby WWTP discharge, or the Naugatuck River mouth site 9B. Milford discharge zone had the highest organic carbon content at 43.3 mg organic carbon/g sediment d.w. and correspondingly had the most PFAA homologues detected and highest total PFAA concentrations measured (8060 pg/g). Milford river sediments were also high in nitrogen (4.7 mg N/g sediments d.w.). The sediments 10m downstream from the Derby WWTP discharge zone display enrichment in organic carbon and nitrogen with respect to the sediments collected in the upper section of the river, approximately 650 m from the effluent pipeline, with sediment organic carbon of 27.2 mg OC/g d.w. and nitrogen 2.5 mg N/g d.w., both increased by a factor of 6 compared to upper river sediments (4.5 mg OC/g d.w. and 0.4 mg N/g d.w.). The enrichment of sediments in organic carbon and nitrogen within the close proximity of a WWTP effluent discharge zone is as would be expected, and reflects settling of effluent derived particulates as well as local biotic uptake, deposition and remineralization of effluent derived dissolved carbon and nitrogen. PFAAs in both SPM

and bed sediment phases in this location indicate the potential for PFAAs to be lost to the sediments once discharged to receiving waters.

Overall, PFAAs with perfluoroalkyl chain lengths ranging from 4 to 9 perfluorinated carbons were detected in the surface waters along the Housatonic River and estuary, with mass flow exhibiting conservative mixing behavior. Longer chained PFAAs (congeners with more than 10 perfluorinated carbons) were detected only in the sediments. For PFCA congeners with between 7 and 9, and PFSAAs with between 6 and 8 perfluorinated carbons (PFOA, PFNA, PFHxS and PFOS), a small but substantial fraction of the mass influx is lost to the sediments within close proximity to the discharge source, however, the major portion of these aqueous phase PFAAs appear to remain in solution once in receiving waters.



Figure 4.30: Concentrations of PFAAs (pg/g) in sediments along the Housatonic River Estuary. (Note: The detection of PFHxS at Station 1 was not duplicated).

Table 4.9: Concentrations of PFAAs detected in the bed sediments collected directly downstream (approximately 10 meters) from the WWTP discharge point. Samples obtained October 2014. (nd=not detected). PFBS, PFDoA, PFTTrA and PFTeA were not detected. Long chain PFCAs were likely lost during SPE WAX sample clean up.

Sampling location	PFAA	Water (ng/L)	Sediment (ng/kg)	Sediment/water ratio
10m downstream				
Milford WWTP	PFBA	1.9	nd	
	PFPA	9.2	nd	
	PFHxA	13.5	nd	
	PFHpA	3	80	27
	PFOA	6.1	820	134
	PFNA	nd	520	
	PFDA	2.9	1160	400
	PFUnA	nd	1690	
	PFHxS	nd	nd	
	PFOS	12.5	3790	303
	PFDS	nd	340	
10m downstream				
Derby WWTP	PFBA	2.1	nd	
	PFPA	5.9	nd	
	PFHxA	9.3	nd	
	PFHpA	3.2	nd	
	PFOA	6.0	nd	
	PFNA	nd	nd	
	PFDA	1.1	380	345
	PFUnA	nd	1450	
	PFHxS	0.3	nd	
	PFOS	5	5060	1012
	PFDS	nd	630	

Concentrations of PFAAs in the sediments in the upper river locations were higher than those in the estuarine sediments, consistent with an upper river dominant source, and/or dilution with low PFAA concentration waters. PFOA and PFOS concentrations measured in the sediments near the Derby and Milford effluent sources are approximately 10 times greater than those measured in the Roter Main River downstream of a WWTP by Becker *et al.* (42). The suite of PFAA homologues detected were however comparable to those reported in riverine sediments in the Netherlands (56), in the Orge River in France (24) Laizhou Bay (57), China and in the Coosa River, Georgia, USA (58), though in this latter study PFOS concentrations close to the suspected source reached a maximum sediment concentration of 20.18 ng/g (see Table 2.8). The estuarine sediment PFAA measurements in this study are higher to those reported in the coastal sediments from Tokyo Bay, Japan, by an order of magnitude (53), but are however similar to the concentrations reported for San Francisco Bay (59).

Maximum sediment PFOS concentrations measured in this study were downstream of the Derby WWTP (5.1 ng/g) and Milford WWTP (3.8 ng/g). These values are much lower than the predicted no effect concentration (PNEC) for PFOS in terrestrial soils of 373 mg/kg d.w. No PNEC values have been determined for riverine and marine sediments, but are derived using the established partition coefficient by Higgins and Luthy (2) for PFOS of 3.0, and the PNEC in freshwater (25 µg/L) or marine water (2.5 µg/L) (47); concentrations of PFOS in this study were below the derived PNEC of 2500 ng/g, however the PNEC value derived does not account for the bioaccumulative properties of PFOS, nor is there any data regarding the PNEC of other

long-chain PFAAs detected in the sediments in this study, including C₁₀-C₁₄ PFCAs detected in the sediments of the marsh situated at the Housatonic River site 3.

The partition coefficients for the distributions of PFAAs between bed sediment and the dissolved phase of the overlying water column were calculated as previously for SPM. The sediment log K_d and K_{oc} values for PFOA, PFNA, PFHxS and PFOS detected in the Housatonic River summer low-river discharge survey are given in Table 4.10. The water-sediment log K_{oc} values obtained for PFOS were approximately an order of magnitude lower than SPM log K_{oc} values, 4.3 ± 0.5 compared to the log K_{oc} derived for SPM, 5.2 ± 0.5 . An equivalent trend was observed by Ahrens *et al.* (25) in a study on SPM and sediments in Tokyo Bay, where the log K_{oc} values derived for PFOS between water and SPM were 4.8 ± 0.1 , and for water-sediments, log $K_d = 3.8 \pm 0.1$. The log K_d partitioning constants for PFOS derived by Ahrens *et al.* of 3.7 for SPM and 2.1 for sediments are consistent with the average values of K_d 's determined in this study, of 3.8 ± 0.3 and 2.3 ± 0.2 for PFOS associated with estuarine SPM and sediments respectively. A similar scale increase in log K_{oc} values for PFOA was found in this study (3.7 ± 0.2 for sediment derived and 4.4 ± 0.1 for SPM derived). Ahrens *et al.* report a larger (1.6 log unit) increase for PFOA (1.9 ± 0.1 for sediment and 3.5 ± 0.1 for SPM derived log K_{oc} values); in this study however PFOA was detected only in the regions of the upper river unlike the saline Tokyo Bay study by Ahrens *et al.*

Partitioning constants derived for the PFAAs detected in the marsh sediments at HR station 3 increased with increasing perfluoroalkyl chain length for PFCAs, however there was no increase between the K_{oc} 's derived for the two PFSA's detected, PFHxS and PFOS. Sediment-water distribution constants (log K_d) for the upper Housatonic

sites are given in Table 4.11, and are also seen to increase with increasing perfluoroalkyl chain length, consistent with the literature. For the Milford site sediments, the organic carbon partitioning $\text{Log } K_{oc}$ increased by an average of 0.48 log units per fluoroalkyl moiety. Salinity was reported by Pan and Yu (26) to increase PFOS partitioning to bed sediments in the Yangtze River; K_d values were reported to increase by a factor of four from 0 – 3.5 ppt. In contrast, comparison of the partitioning constants derived in this study for the sediments located in Derby and Milford do not show any increased PFDA or PFOS partitioning with increased salinity. The trend of increasing pH down river, from the fresh Derby site to the saline Milford site, is a plausible factor to explain the higher K_{oc} values in the Derby sediments, unfortunately the pH of the Derby water was not recorded therefore it is not possible to quantify the potential pH effect.

The $\text{log } K_{oc}$ value derived for PFOS sediment-water distribution at the freshwater site Derby was greater than that in Milford (4.58 compared to 3.83). This value is likely a reflection of the relatively high aqueous PFOS concentrations that are emitted from the Derby WWTP (Table 4.3), the higher PFOS-SPM concentrations in the Derby effluent, (Appendix Table A7, plant C) and the design of the discharge pipeline, which is a simple opening on the eastern bank of the river and so effluent is therefore unlikely to mix with the whole body of the river water at this location, leading to increased PFOS sedimentation in the proximity of the discharge zone, particularly under low river flow hydrology. The highest K_{oc} value for PFOS was found in the LIS station 4P, however scale of this coefficient may have been inflated due to the low organic carbon content of the sediments in this location since the K_d is closer in value to the that seen at LIS 3P.

Table 4.10: Log K_d and K_{oc} values (\pm range of duplicate samples) calculated for PFAAs detected in both bed sediments and overlying waters at different sites along and around the Housatonic river mouth (survey 1, July 2012). River 1 was not included as the data for PFHxS was not duplicated.

Sample station	PFOA	PFNA	PFHxS	PFOS
K_d				
River 3marsh	2.20 \pm 0.02	2.36 \pm 0.07	2.58 \pm 0.04	2.55 \pm 0.02
LIS 3P	1.86 \pm 0.09			2.35 \pm 0.08
LIS 4P				2.13 \pm 0.08
Sample station	PFOA	PFNA	PFHxS	PFOS
K_{oc}				
River 3marsh	3.85 \pm 0.06	4.02 \pm 0.12	4.24 \pm 0.03	4.20 \pm 0.04
LIS 3P	3.66 \pm 0.09			4.14 \pm 0.07
LIS 4P				4.79 \pm 0.10

The combined organic carbon partitioning values for each PFAA detected in both sediment and water phases along the Housatonic River estuary, for all surveys, result in an average log K_{oc} value for PFOA of 3.7 and for PFOS 4.3. Laboratory based sorption experiments report average log K_{oc} values for PFOA and PFOS to be around 2.8 and 3.0 respectively, therefore as with the log K_{oc} values derived for PFAA SPM partitioning, sediment partitioning coefficients derived in this survey are higher than literature values. The average log K_{oc} values for the Housatonic River estuary are in close agreement with those published by Ahrens *et al.* (25) for sediment partitioning in Tokyo Bay; values

from their study were not only directly comparable, but also increased with increasing perfluoroalkyl chain length to the same extent (Figure 4.31). The log unit increase per perfluoroalkyl moiety for the sediment derived log K_{oc} values in this study is 0.43, consistent with that determined by batch sorption experiment of 0.50-0.60, as reported by Higgins and Luthy (2). The data shown by the investigation into PFAA distributions in riverine and estuarine sediments in this study confirm that field-based partitioning coefficients are of greater magnitude than those derived under laboratory conditions; however the extent to which the hydrophobic chain controls partitioning is consistent.

Table 4.11: Log K_d values (\pm range of duplicate samples if $n=2$, or no range given if $n=1$) calculated for PFAAs detected in both bed sediments and overlying waters 10m downstream from WWTP effluent discharge zone. Samples obtained October 2014 (survey 3).

WWTP	Salinity	PFHpA	PFOA	PFDA	PFOS
Derby	0.1 ppt			2.56 \pm 0.23	3.00 \pm 0.03
Milford	9.0 ppt	1.43	2.13	2.60 \pm 0.05	2.48 \pm 0.16
Derby	0.1 ppt			4.13 \pm 0.17	4.58 \pm 0.01
Milford	9.0 ppt	2.75	3.46	3.97 \pm 0.09	3.83 \pm 0.18

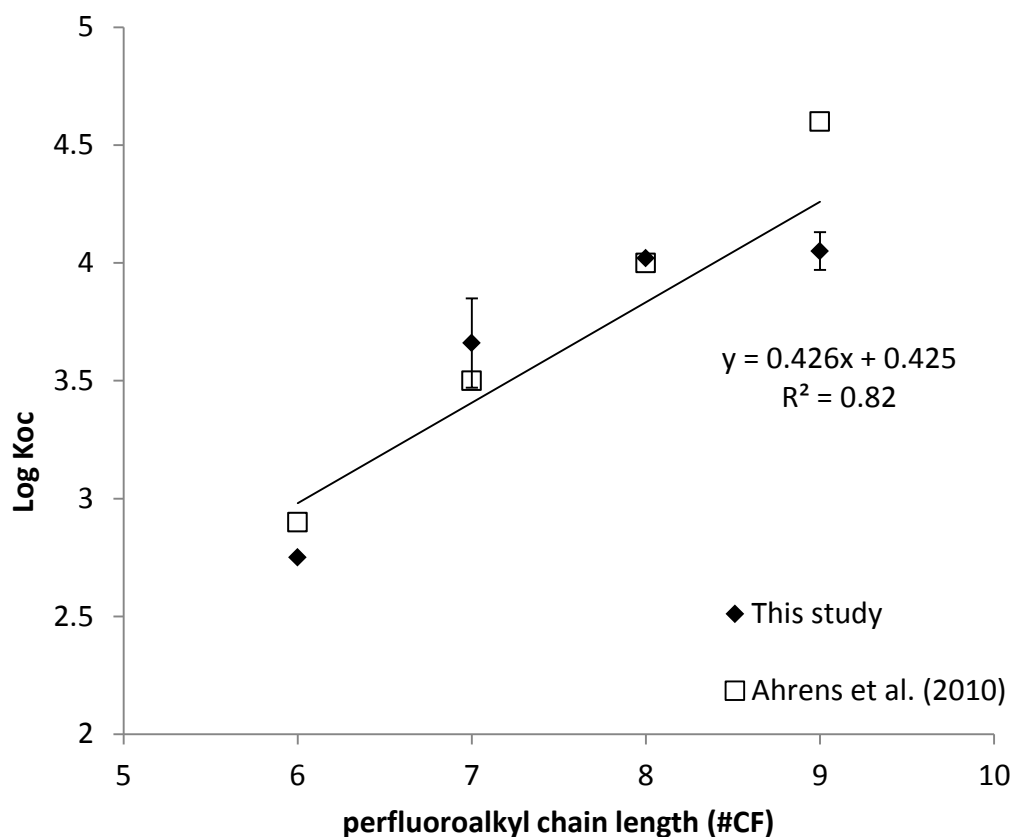


Figure 4.31: Increasing partitioning to sediments with increasing perfluoroalkyl chain length observed with PFCAs in Housatonic River estuary. Error bars given are the range for samples with $n > 1$; for PFHpA and PFNA $n = 1$, for PFOA $n = 3$ and for PPFDA $n = 2$. Regression equation given is for the data from this study.

In conclusion, the analysis of solid-water PFAA distributions in this study provides useful information into the processes that control the fate of PFAAs in riverine and estuarine aquatic environments. Though the major portion of the PFAA mass flux, consisting for PFAAs with 4-8 perfluorinated carbons, is conserved along the river and estuary, a small quantity of longer chained PFAAs (with perfluoroalkyl chain lengths for 6 or greater) are lost to sediments, particularly in the region directly downstream from

WWTPs and in the organic carbon rich marsh zones in the mixing region of the river-estuary. PFAAs with greater than 10 perfluorinated carbons were detected only associated with the sediments. Therefore PFAA contamination in these organic carbon rich environs could be adversely affecting benthic organisms; particularly since sediment bound PFAAs have been shown to be both bioavailable (60) and a major source of PFAAs to an aquatic food-web (61).

4.4.6 PFAAs in oysters

Bivalves are useful sentinel organisms for use in pollutant monitoring due to their reliance on suspended particulates, to which hydrophobic organic contaminants adhere, as a supply of food, and due to their large volume filtering capacity (62). They can be deployed deliberately into an area of interest for a regulated amount of time where they filter continuously, providing an integrated collection of contaminant, or can be harvested from naturally occurring beds in the area of interest. The location at which bivalves can be deployed is limited by the salinity range in which they will remain alive and healthy.

A small pilot study was conducted in the Housatonic River mouth utilizing oysters as a biomonitoring species, in order to compare to PFAA concentrations measured in river surface waters, suspended particulates and bed sediments, and to calculate bioaccumulation factors (BAFs) for any PFAA detected in both oyster and water phases. Live deployments were limited to the saline environs of the Housatonic River mouth located in the vicinity of HR stations 2 and 3 which is the natural salinity range for these

organisms. It must be noted that the oysters in this study were deployed in or collected from regions in the Housatonic that are designated as no shellfish areas, and are not in any proximity to commercial or recreational shell-fishing zones. Data from this pilot study does not in any way indicate any contamination of commercial or recreational oysters, as these latter beds are located in regions away from and separate to any effluent or riverine derived impact regions.

Oysters were harvested from the Poquonnock River (eastern Connecticut) near a natural area preserve, and placed into nets which were deployed at two sites; Brewers Marina in Stratford located on the shore close to HR sampling station 3, and at Knapps landing dock, located also in Stratford, in the proximity of HR sampling station 2. Oysters were deployed for 10 days, from July 7th to July 27. During the HR survey of July 24th, oysters were also harvested from the rocky bed on the river floor at HR sampling station 2. Concentrations of PFAAs measured in whole oyster samples is given in Figure 4.32. Only two oysters were extracted from each site as a test for future work and though the data presented here is pertinent to the fate and transport of PFAAs in the HR, it is only preliminary.

Similarities in both the PFAA concentrations and composition profiles between the deployed oysters (Brewers and Knapps) with the oysters harvested at HR station 2 indicate that the live deployed oysters were healthy and actively filtering for the duration of the 10 day deployment, and that PFAAs detected are consistent with PFAAs in the Housatonic study site. Long chained ($> C_8$) PFAAs were detected in the oysters, no shorter chained PFAAs were detected though present in water samples, consistent with the fact that only longer chained PFAAs are bioaccumulative, or associated with SPM.

PFOA and PFTeA were the most prevalent PFAAs, detected in 100% of samples; PFOS, PFDS and PFDoA were detected in 83.3%. PFNA was not detected in oyster tissue however, but was detected in 100% of river water samples. PFDoA and PFTrA were detected in the highest average concentrations, at 1670 and 1645 pg/g-dry mass respectively. This last result highlights the importance and usefulness of bio-monitoring studies in assessing the impact of pollutants in a region, since the longer chained PFCAs were not detected in water samples at all, mostly due to poor SPE recoveries (<50%) for the large volume river water (2 L) extractions.

Partitioning constants for PFOA and PFOS, detected in both oyster tissue and surrounding water, were derived from this data. The deployed oysters were compared to surface waters, whereas the oysters from the bed region at HR station 2 were compared to deep water samples obtained during the low flow survey July 24th. The equilibrium partitioning between the oyster and overlying water, $K_{oyster,w}$ was calculated using;

$$K_{oyster,w} = \frac{ng(PFAA)/kg(dry\ mass)}{ng(PFAA)/L(water)}$$

Log $K_{oyster,w}$ values for PFOA and PFOS for the oysters obtained from the river bed were 2.53 and 2.86 respectively, and are in direct comparison to the log K_d values obtained for the sediments obtained from the organically rich salt marsh area at station 3, 2.20 and 2.55 for PFOA and PFOS. Log $K_{oyster,w}$ derived for PFOA and PFOS detected in the oysters deployed in surface water sites were generally slightly higher; PFOA = 2.94 (2.77-3.19 range, n=4) and PFOS = 2.90 (2.75 – 3.12 range, n=3).

Bioaccumulation (BAF) describes the net uptake of a pollutant from the surrounding environment by all possible routes, such as dietary, dermal, respiration, and are analogous to partitioning constants, $K_{\text{oyster,w}}$, but consider the wet mass of the aquatic organism:

$$BAF_{Total} = \frac{ng(PFAA)/kg(wet\ mass)}{ng(PFAA)/L(water)}$$

BAF values > 1 indicate that the accumulation in the organism is greater than the surrounding water. BAF values for this data set for PFOA are 31.5 for deep and 71 for surface water oysters, and for PFOS, 66.5 for deep water and 68 for surface water oysters. The higher BAF values for surface compared to deep water oysters may be attributable to the greater exposure to PFAAs from higher concentration up-river waters, however as this data set is limited, interpretations are only tentative.

For filter feeding organisms like oysters, ingestion of contaminated particulate matter is one of the major routes of PFAA bioaccumulation (60), and those near to source sites have elevated amounts (57). Given the close correlation to the K_d values obtained for sediments in this study site, the bioaccumulation of PFAAs in these oysters may be a reflection of the amount of PFAAs ingested via contaminated particulates. Comparison of PFOS/PFOA ratios in surface and deep waters, oysters and in the bed sediments at HR station 3 show that the PFOS/PFOA ratio in the river bed oysters (2.7) is similar to the PFOS/PFOA ratio in the sediments of station 3 marsh (2.9). The deep water and surface water PFOS/PFOA ratios measured in River station 3 are both 1.5, reflective of a well-mixed water column. PFOS/PFOA ratio in the surface water deployed oysters is 1.2, which is consistent with the PFOS/PFOA surface water signal.

While there is little overlap with the other PFAA homologs between these compartments due to the long-chain PFAAs being detected only in oyster and sediment samples and shorted chain species prevailing in the aqueous phase, a comparison of the PFAA profiles for the river bed oysters and the bed sediments at River station 3 (marsh) and LIS 3P show moderate to good correlations; for the River station 3 marsh $R^2 = 0.56$, $p = 0.053$, and for LIS 3P sediments, $R^2 = 0.94$, $p < 0.01$. The consistency of the PFAA signal in both river bed oysters and in the LIS sediments obtained from station 3P suggests that the Housatonic River could be the source of the PFAAs detected in this region of the LIS. Further research to confirm this observation is warranted.

Detection of the long chain PFAA compounds in the oyster tissues in this pilot study highlights the importance in further research to address the role of SPM in the transport of longer chained PFAAs and sediments as potential sources to biota in this region. Additional assessments should be performed in this region regarding potential sources of and input amounts to the aquatic environment, and levels of longer ($>C_8$) perfluoro-alkyl chain PFAAs to the food chain, as due to their high bioaccumulation and biomagnification potential, these compounds may be exerting negative effects on locally sensitive marine wildlife and ecosystems. Addressing the issue of the lower SPE recoveries of long-chain PFAAs ($> C_{11}$) in conjunction with better tuning of the UPLC-MS/MS to enhance detection of these same compounds would be required in order to determine the concentrations of these PFAAs in WWTP effluent. Finally, the result of this pilot study indicates that further research is merited to address the potential levels that may be in local LIS seafood as consumption of seafood is the greatest source of human exposure to PFAAs (63).

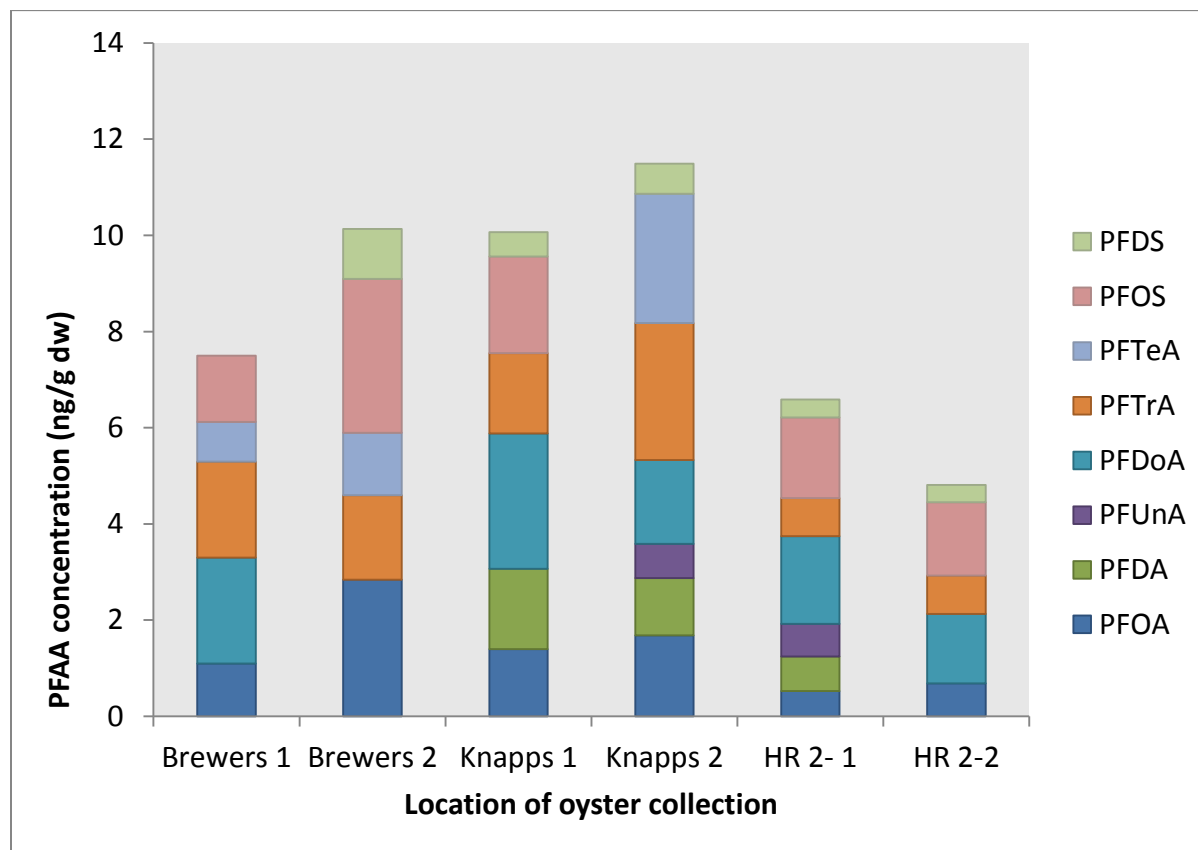


Figure 4.32: Concentrations of PFAAs detected in duplicate oyster samples (2 from each location) in ng/g dry weight.

4.5. Summary

The aims of this study were to: investigate the occurrence and transport of PFAAs in both fresh and saline waters of the Housatonic River (HR) and estuary, under contrasting hydrological regimes, estimate the ranges of mass flux of PFAAs into the LIS and relative significance of WWTP point sources, assess the influence of salinity on PFAA water-SPM and water-sediment biogeochemical dynamics under field conditions,

and gage the role of SPM in the fate and transport of PFAAs. Loading to the river from WWTP point sources and occurrence in surface waters was determined under summer low river flow, and again under high river discharge though the second survey was limited in scale by comparison. Wastewater effluent was found to be a major point source of PFAAs to the HR with, for the majority, concentration and composition profiles that displayed little variation over the course of this study, and are likely the function of a majority domestic waste signal. Using this assumption, the concentration profile of PFAAs in the Naugatuck River could be accounted for to high degree; a result which is particularly useful for regional risk assessment applications. Results from the Housatonic River survey found that approximately 80% of PFAAs detected in the surface waters in the northern section of the Housatonic River study site originated from up-river sources. Further research is warranted to elucidate the sources of PFAAs to the upper Housatonic.

The inverse relationship between river discharge and PFAA concentrations suggests the significance of point sources, such as WWTPs, to this river system, however the three-fold increase in mass flux with the increased hydrology suggests that the contributions from non-point sources cannot be excluded. Important non-point sources that should be considered for future research include run-off and precipitation, which are known sources of PFAAs to the aquatic environment. Additionally, results presented in this study showed that long-chain ($>C_5$) PFAAs can accumulate in the sediments within proximity to the emission sources investigated, namely the effluent discharge of WWTPs. Given the lower aqueous phase PFAA concentrations due to dilution with the high river volume in the spring high-discharge regime in conjunction

with increased turbulence, PFAAs partitioning from sediments into the aqueous phase may constitute an important source. The extent to which sediments act as secondary sources of PFAAs under high-discharge hydrology should be explored in future research.

Investigations into PFAA sediment concentrations determined that although the major portion of the PFAA mass discharged into the river system is conserved and capable of being transported long distances, a small fraction of longer chained PFAAs ($>C_5$) partitions to bed sediments. However, this behavior was only observed to occur within close proximity of effluent discharge zones and within the river-estuary mixing zone; in both cases the sediments where PFAAs partitioned were rich in organic carbon. Salt marsh areas in the mixing zone and in the proximity of the Stratford WWTP effluent discharge pipeline are hypothesized to be catchment areas for longer chain PFAAs. The presence of PFAAs in these organic carbon rich areas may be cause for concern due to potential bioavailability to benthic organisms, particularly given that effluent POM is known to be a high quality food source that is preferentially assimilated by benthic biota (64), and that sediment fraction PFAAs have been shown to be bioavailable (59) as well as a major source of PFAAs to an aquatic food web (65).

Specific focus in this study was directed towards better understanding of the role of suspended particulate matter in the fate and transport of PFAAs in receiving waters, due to the importance of SPM in linking the distributions of contaminants between the water column, the bed sediments and the food chain. PFOA was measured in the SPM phase in samples obtained from 10 m downstream of the effluent discharge sources at Derby and Milford WWTPs. SPM-water K_{oc} values derived were higher by 0.9 log units

than values previously published for SPM in Tokyo Bay, but were however consistent with those derived for efSPM-water partitioning of PFOA, leading to the conclusion that the samples obtained in these locations in close proximity of the WWTP effluent discharge were likely samples of effluent matter and not riverine SPM. Therefore the higher than previously published partitioning coefficients derived for PFOA efSPM in Chapter 3 were verified by these observations.

PFOS was the only PFAA detected in the suspended particulate phase along the salinity gradient of the Housatonic River and estuary, and in the Naugatuck River. PFOS was detected in the SPM phase in 100% of samples obtained along the Housatonic River and in the regional LIS stations during the summer low-flow hydrology. Partitioning-coefficients derived for PFOS water-SPM phase distributions were an order of magnitude greater than that describing water- bed sediment distribution in this study, and were consistent with the value obtained for effluent-SPM in Chapter 3. These results are in line with reports of greater partitioning to SPM in the field, as observed in Tokyo Bay (25) and in the River Seine (28). The average riverine SPM derived $\log K_{oc}$ value obtained for PFOS in this study (5.1 ± 0.5) was similar to that published for Tokyo Bay (4.8 ± 0.1).

While the PFOS - SPM derived $\log K_{oc}$ values obtained in this study ranged over one order of magnitude, they did not vary correspondingly with increasing salinity as predicted, and contrary to the results of previously published studies (44, 45). Results from a laboratory SPM-water partitioning experiment, utilizing end member waters, however confirmed the SPM-water K values for PFOS obtained in the field, and the apparent lack of salinity variation, however field riverine and estuarine SPM were

utilized in this experiment therefore different SPM chemical character may have accounted for the lack of salinity effect on K_{oc} values.

As this is the first time the PFAAs have been measured in this region, the results from this study serve to provide a baseline on the occurrence and distribution behaviors of PFAAs in the HR and by extension, in LIS. While shorter chain PFCAAs such as PFPA were found to be a major component in both effluents and river waters, indicative in a shift towards eliminating longer ($>C_8$) PFAAs in products and applications, a major polluting event detected in the Stratford WWTP effluent in the spring 2013 survey illustrates that longer chain PFSAAs are still in use and associated with specific industry applications, such as airports and aviation industries. Longer chain PFAAs were also detected in the Housatonic estuary by the use of oysters deployed as biomonitoring samplers, which implies an important role of SPM in the fate and transport of these less soluble PFAAs since these will be more likely associated with the SPM phase, as was shown previously in effluent SPM (Chapter 3). These results lead to the conclusion that further research should be concerned with the presence of these bioaccumulative and biomagnifying longer chain PFAAs in biota and sensitive wildlife species in the region, as well as an assessment made towards potential levels in seafood and the possible risk for human exposure.

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Occurrence of Phthalate Acid Esters in Wastewater Effluent and Housatonic River Receiving Waters

Abstract

Phthalic acid esters (PAEs) are a class of organic compounds that have been recognized as ubiquitous environmental contaminants with the potential to cause serious adverse effects to wildlife and to humans. Of the 4.5 million tons of PAEs manufactured each year, approximately 50% is di-(2-ethylhexyl) phthalate (DEHP) used chiefly in the manufacture of PVC. The occurrence and partitioning behaviors of six phthalates; dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DnOP) were investigated in the final effluents of several Connecticut (CT) wastewater treatment plants (WWTPs) in the spring of 2012. DEP and DEHP were the most prevalent PAEs detected in wastewater effluent. Based on the average concentrations measured, and the reported total average daily flux of 3.3×10^9 L treated

effluent entering the LIS watershed, the potential daily mass flux of DEP and DEHP to the LIS was estimated at 10 - 140 g/day and 170 - 750 g/day respectively.

A second survey conducted in the summer of 2012 investigated the occurrence and distributions of the target PAEs in the final effluent from six WWTPs located along the Housatonic River (CT), and the subsequent impact of the receiving waters of the river and estuary. WWTPs were shown to be sources of PAEs to the Housatonic River. Surface waters in the estuarine region of the Housatonic River mouth had greater PAE concentrations compared to the riverine waters, indicating that the estuary could be a trap for these organic contaminants. Suspended particulate matter and dissolved organic matter was found to play a significant role in the transport of PAEs in wastewater effluent, river and estuary waters.

5.1 Introduction

The global transport and fate of several phthalic acid esters (PAEs) has received widespread attention in recent years due to compounds in this class identified as endocrine disrupting chemicals (EDCs). The potential impact of PAEs on human and ecosystem health has been recognized in the scientific community, with reports in media also drawing the attention and concerns of the general public. During the past 40 years, the high production volume of PAEs (mainly in the production of PVC and the numerous commercial uses of) has led to their ubiquitous presence as environmental pollutants, even in remote marine environments. Wastewater treatment plants (WWTPs) are known sources of PAEs to the aquatic environment however their presence and estimated loadings to the LIS from WWTPs located on or near the Connecticut

shoreline has never been reported. The overarching objective of this research was to determine the occurrence and partitioning behaviors of 6 PAEs in wastewater effluents and in the receiving waters of the Housatonic River and Estuary.

Wastewater effluent discharge has been identified as a major source of PAEs to the aquatic environment (1) however no data has been published in the peer-reviewed literature on the presence of PAEs in wastewater discharges from CT WWTPs located on or near the LIS shoreline. The occurrence of PAEs in the receiving waters of the Housatonic River and estuary downstream from several of the WWTPs was also investigated to provide a preliminary evaluation of the impact of PAEs to the water quality of the LIS. An important factor in determining the transport and fate of organic pollutants is the distribution between the dissolved, colloidal and particulate phases (2). DOC concentration has been reported to correlate significantly with organic pollutant partitioning, suggesting it plays an important role in transport and in decreasing the efficiency of WWTPs in removing hydrophobic contaminants (3). Additional aims of this study included investigating the partitioning of PAEs between apparent 'dissolved' phase and the suspended particulate matter phase in both WWTP effluent water, and in receiving river and estuarine waters, and to investigate the role of DOC, salinity and SPM composition on partitioning.

This chapter begins with a brief topic overview of PAEs followed by the results obtained in the investigation of the occurrence of PAEs in several CT WWTPs and in the receiving waters of the Housatonic River.

5.2 An overview of Phthalate Acid Esters

Phthalic acid esters (PAEs) are a class of organic compounds that, over the past ten years, have increasingly been recognized as ubiquitous environmental contaminants that have the potential to cause serious adverse effects to wildlife and to humans. These chemical compounds include an array of molecule structures, with alkyl chain lengths ranging from 1 to 13 carbons, a corresponding eight orders of magnitude increase in log octanol-water partition coefficients (K_{ow}), and a four order decrease in magnitude of vapor pressure (4). There are eighteen commercially important PAEs, widely used for the past 40 years as additives to products such as plastics and resins, due to their high stability, fluidity, high melting and boiling points, and low volatility. Most phthalate esters are used as plasticizers in the manufacture of PVC, making the plastic softer and more flexible. PAEs are also used in other resins such as polyurethanes, poly vinyl acetate, epoxy resins and cellulose esters. In some plastics, PAEs may constitute from 35% to 50% of the total weight (5, 6). Plastics find uses in numerous applications, including as building materials, home furnishings, food packaging, detergents, pesticide formulations, personal care products and medical products (7). The PAEs themselves are not chemically bound to the final products, so are able to leach out continuously or be released into the air. As a result PAEs are widespread contaminants; found in food, which is a major source of exposure to humans, as well as indoor and outdoor environments (8). Of the 4.5 million tons of PAEs manufactured each year, approximately 50% is di-(2-ethylhexyl) phthalate (DEHP) (5), used chiefly in the manufacture of PVC. Other commercially important PAEs are dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butylbenzyl phthalate (BBP) and

di-n-octyl phthalate (DnOP). The molecular structures of these compounds, which are the target PAEs in this study, are given in Figure 5.1.

In comparison to known persistent organic pollutants polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) in Corpus Christi Bay (Texas), PAEs were determined to be the most prevalent pollutant in both water and sediment samples (9). In addition, DEHP and DnBP have also been detected in remote areas, far removed from industrial and human activity, such as in the atmosphere and precipitation on the Enewetak Atoll in the North Pacific Ocean (10). In our local environment, samples of streambed sediment obtained from coastal basins in New England (1998-1999) show DEHP to be most prevalent PAE contaminant, with concentrations ranging from 0.15-11 $\mu\text{g/g}$, BBP range of 0.01-0.5 $\mu\text{g/g}$; DBP range of 0.01-0.1 $\mu\text{g/g}$; DOP concentrations of 0.1-0.7 $\mu\text{g/g}$ and DMP and DEP detected infrequently at low ($<0.1 \mu\text{g/g}$) concentrations (11)

Previous studies, sponsored by the Chemical Manufacturers Association Phthalate Esters Program Panel in 1985, concluded that 14 commercially important PAEs had low potential for adverse environmental effects following acute toxicity tests on nine representative species, chronic reproduction studies with the crustacean *Daphnia magna*, biodegradation testing, and physicochemical properties (12). However, a study published in 1971 found that exposure of *Daphnia magna* to 3 $\mu\text{g/L}$ of DEHP (environmentally relevant concentrations), resulted in a significant decrease in growth and reproduction (13). Several phthalate esters have been classified as endocrine disrupting compounds (EDCs). The suspected (anti)-estrogenicity is due mainly to *in vitro* assay investigations. These include receptor mediated reporter gene assays and

cell proliferation assays, where the following PAEs (in decreasing potency) BBP > DBP > DEP are reported to elicit estrogenic responses (14). PAEs are also suspected (anti)-androgens; DEHP, BBP and DBP, as determined by *in vitro* studies, and *in vivo* studies with specific androgenic endpoints following *in utero* exposures resulting in adverse male reproductive effects (15, 16). Anti-androgenic effects may disrupt the proper differentiation and formation of male sex organs if exposure occurs during critical windows of development, such as in the first trimester of human pregnancy. Significant associations have been reported between maternal urinary phthalate and phthalate metabolite concentrations and shorter anogenital distance in male infants (17). Reproductive dysfunction was also reported in marine medaka (*Oryzias melastigma*) following in chronic exposure to DEHP, including the induction of liver vitellogenin in male fish, and altered gonad histology (18).

Traditional toxicological testing regimes obtain Tolerable Daily Intake (TDI) values by determining acutely toxic doses, then reducing the concentrations until finding no observable adverse effect levels (NOAELs). However, with endocrine disrupting compounds, this approach is insufficient. The endocrine system is a complex, physiologically integrated system that uses chemical signals (hormones) to regulate internal functions of the body. EDCs can alter biological functions through a variety of different mechanisms; by mimicking the hormone and binding to their natural receptor, either as agonists or antagonists, by altering synthesis or breakdown of natural hormones, or by modifying production or function of hormone receptors. These effects occur naturally at very dilute concentrations (ppm-ppt) and hormones are exceptionally potent compounds; the hormone-receptor binding initiation mechanism sets off a

domino-effect cascade of cellular reactions. Biological responses are also much greater at lower concentrations, as at higher concentrations, cells respond by, for example, reducing the number of receptors, producing a saturation-inhibition response. Therefore, using traditional NOAELs, the lowest concentrations with the maximal responses would not be determined.

As endocrine signals during fetal gestation initiate a programmed chain of irreversible developments in reproductive and neurological systems, there is a very real public and wildlife health concern regarding the developmental and/or reproductive toxicity of suspected EDCs such as phthalates. PAEs have been reported to adversely affect the reproductive capacity and impair development in marine aquatic organisms, causing genetic aberrations in mollusks, crustaceans and amphibians at environmentally relevant exposure concentrations in the low ng/L to µg/L range (19, 20).

Physical-chemical properties

PAEs are the di-esters of 1,2-benzenedicarboxylic acid, or ortho-phthalic acid. The molecular weights of the seven selected PAEs range from 194.2 – 418.6, and alkyl chain lengths vary from 1 to 9 carbons (Figure 5.1). The abbreviation (n) indicates a linear isomer (DnBP, DnOP). BBP is non-symmetrical, having a butyl and a benzyl alcohol ester group. DEHP is a pure isomer, however the longer chain (>6) molecules are usually present as isomeric mixtures in commercial preparations. A summary of physio-chemical properties of the six PAEs, on which this study is focusing, can be found in Table 5.1. Although the literature values for the physico-chemical properties of each PAE may vary by many orders of magnitude (13), the general trends across the

group of selected PAEs are consistent. Each of the six PAEs is a liquid at ambient temperatures, and each has a high boiling point.

Solubility is an important parameter for pollution studies, as this can be an indication of aquatic bioavailability and thus toxicity of a chemical species, as well as absorption and bioaccumulation potential. Aqueous solubility also determines the routes and sources of contamination, since pathways from wastewater or landfill leaching involve movements of water. Moderate to high solubilities have values >1 mg/L, hydrophobicity is noted where values <1 mg/L. There is a general trend of decreasing solubility with increasing alkyl chain length within the group of selected PAEs, the shortest chain species, DMP, being the most soluble. The high molecular weight PAEs (DnOP and DEHP) are hydrophobic, but are also less dense than water, therefore it is possible to predict that these PAEs may be present in the micro-layer at the air-water interface.

There is a well-established inverse relationship between K_{ow} and water solubility, which is seen in the values of the PAEs presented in Table 5.1. The higher molecular weight PAEs being most hydrophobic. Preference for the octanol phase indicates that the longer chain PAEs are more likely to partition into soil or sediment organic matter; high K_{ow} values are indicative of strong sorption to dissolved organic carbon (21). The high K_{ow} values are also a predictor of the tendency of a chemical to partition into animal lipids, pinpointing the potential for bioaccumulation in aquatic organisms such as fish, filter feeders and other sediment residing organisms.

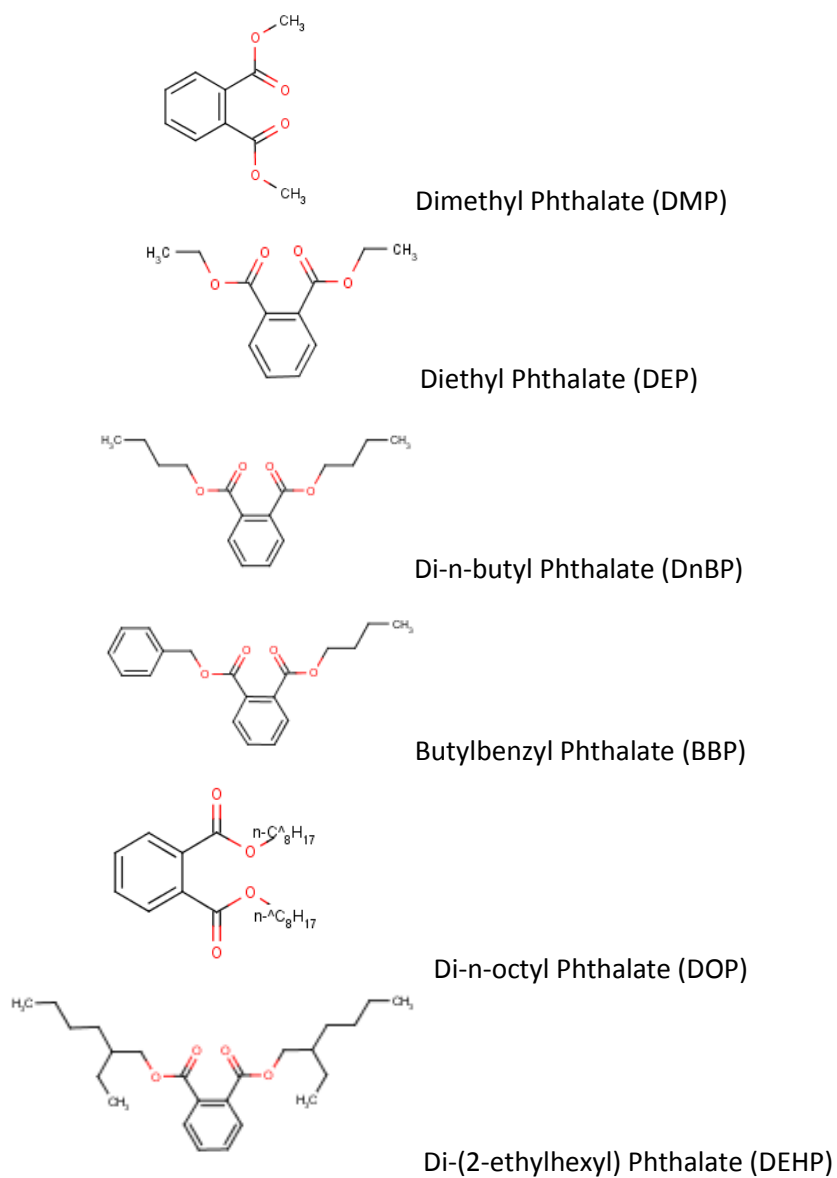


Fig. 5.1: Molecular structures of six target PAEs

Table 5.1: Physical Properties of six phthalate esters (Data obtained from (4, 21) and from ChemID Plus [<http://chem.sis.nlm.nih.gov/chemidplus/>] <accessed 5/25/15>) (NA = not available)

Phthalate	CAS # Formula	Alkyl chain length	M. wt	M pt B pt (°c)	Specific gravity (20 °c)	Log K _{ow}	Solubility (mg/L)
Dimethyl (DMP)	131113 C ₁₀ H ₁₀ O ₄	1	194.2	5.5 283.7	1.192	1.61	4200
Diethyl (DEP)	84662 C ₁₂ H ₁₄ O ₄	2	222.2	-4. 295	1.118	2.54	1100
Di-n-butyl (DnBP)	84742 C ₁₆ H ₂₂ O ₄	4	278.4	-35 340	1.042	4.28	11.2
Butyl benzyl (BBP)	85687 C ₁₉ H ₂₀ O ₄	4, 6 (aromatic ring)	312.4	-35 370	1.111	4.70	2.7
Di-n-octyl (DnOP)	117840 C ₂₄ H ₃₈ O ₄	8	390.6	-25	0.978	7.73	0.02
Di-(2-ethyl- hexyl) (DEHP)	117817 C ₂₄ H ₃₈ O ₄	8	390.6	-47 384	0.986	7.73	0.27

A number of earlier studies examining the dissolved versus suspended particulate matter-bound (SPM) fraction of PAEs in surface river water samples (summarized by Staples *et al.*, (4)) found that the high molecular weight PAEs were mostly particle bound, whereas for the lower molecular weight species (DMP-BBP) highest percentages of total concentration were in the dissolved fraction (Table 5.2).

Table 5.2: Results from several studies of the water-solid partitioning behavior of a number of PAEs in river water samples. (Compilation of data given in (4))

PAEs Studied	% Dissolved	% SPM
DnBP	86	14
DEHP	47	53
DnBP	98	2
DEHP	33	67
DMP-BBP	83-85	15-17
DEHP- DnOP	26-48	53-74

Neutral, hydrophobic molecules exhibit different partitioning activities in the saline environment of estuaries compared to the fresh water of rivers. Being both the most prevalent PAE in the environment, and a particularly hydrophobic species, the behavior of DEHP in fresh and saline environments is of significant interest. Adsorption to particulate matter and subsequent deposition is a likely fate for DEHP in rivers, estuaries and coastal zone areas receiving such wastes. DEHP has a relatively lower solubility in sea water than in distilled water due to electrorestriction (22) and exhibits increased sorption capacity with increased concentrations of dissolved salts (22, 23).

Salting out of the hydrophobic DEHP is also seen by the significantly enhanced adsorption onto estuarine particles in sea water versus river water (22). In addition, the particle-water distribution coefficient exhibited a strong inverse relationship with estuarine particle concentration (22) and with sediment concentration (23), so that the sorption capacity of the particulates is reduced with increasing particulate concentrations. It is possible that this is due to some particle-particle interaction

mechanism such as flocculation (increased particulate aggregation that would result in reducing the surface area available for adsorption). The authors (22) point out that this is a likely mechanism, since flocculation is inhibited at higher salinities, and the data presented suggested that the sensitivity of adsorption to particle concentration was lower in saline water.

PAEs in the aquatic environment

Concentrations of DBP and DEHP have been reported for various rivers in Germany. As the concentrations of these PAEs were 2 or 3 orders of magnitude greater than those found in the North Sea; the authors conclude that river borne contaminants are a significant source in this region (7). DEHP, BBP, DnBP and DEP (in order of high to low concentrations) was detected in numerous waste water samples in Oakland, CA (24), from residential and industrial sites, allowing the authors to conclude that some major PAE sources to wastewater include industrial laundry, pharmaceutical and adhesives manufacturers. Spatial characterization investigations of the Houjing River, Taiwan determined that a likely discharge source of DEHP is the effluent from Formosa Petrochemical Corporation (25).

In addition to point sources, other potential sources to the aquatic environment of PAEs are solid plastic wastes, which are either dispersed throughout the marine environment or confined to landfills through which water percolates. A study undertaken in Germany examined the potential contamination of PAEs from domestic plastic waste by investigating the composition of refuse, and from the results of experiments simulating leaching (26). Results indicated that the lower molecular weight PAEs (DMP

and DEP) were leached to a greater extent than DBP, BBP and DEHP which is an reflection of the higher solubilities of DEP and DMP.

The discharge of effluent from WWTPs is considered the primary source of PAEs to the aquatic environment (1, 27, 28, 29) Mass balance studies find that the WWTP process does remove a substantial portion (> 90%) of PAEs from the waste stream via partitioning to solids and settling or by microbial degradation, (30, 31, 32). However, substantial levels of PAEs can be detected downstream of WWTPs (27, 33). As in effluents, DEHP then DnBP were the predominant PAE in surface samples of the River Seine, France (33), in the Netherlands (5), and receiving waters in Germany, with large variations, over two orders of magnitude, observed (29). Furthermore, due to the increased salinity and turbidity influencing the relative solubilities and sorptive behavior, as well as particulate flocculation and settling mechanisms, estuaries are considered to act as a trap for hydrophobic organic contaminants such as PAEs. As such, PAEs are often detected in elevated concentrations in estuaries (22), with reported concentrations in the range of 0.1-300 µg/L for surface marine waters, and 1.0–13.5 µg/L for freshwater sites, as summarized by Xie *et al.*, 2005 (7). However, the concentrations of DEHP in marine waters of the Netherlands were reported to be of the same order of magnitude as those in freshwaters (5).

The aims of this study were to assess the occurrence and distribution behaviors of PAEs in wastewater discharges from several CT WWTPs impacting the LIS and major tributaries, and to determine the presence and distributions of PAEs in the downstream fresh waters of the Housatonic River and saline waters of the estuary, in order to provide a preliminary evaluation of the impact of PAEs in the CT shoreline

region. Finally, to investigate the role of SPM and DOC in the fate and transport of PAEs in final effluent, riverine and estuarine waters, and derive field based values for partitioning parameters.

5.3 Methods

5.3.1 Sample Collection

12 WWTPs located along the Connecticut shoreline of the LIS, or on the major tributary rivers, the Connecticut River and the Housatonic River, were surveyed in spring and summer of 2012. Samples of final effluent collected from 11 WWTP in spring 2012 were either grab or 24 hour composite samples, as detailed in Table 5.3. Additional WWTP final effluent samples from the 6 WWTPs located along the Housatonic River (HR), 5 of which had been previously sampled in the spring, were collected July 17th, one week prior to collection of Housatonic River and LIS surface water samples (Table 5.4). Samples of river water were obtained from the Housatonic River from shore or from on board the *R/V Lowell Weicker*, July 24th, 2012, and from the LIS on July 25th, 2012, as detailed in Table 5.5. Effluent water was collected in 1 L amber glass bottles which had been previously solvent rinsed and baked at 450 °C. River waters were collected in 4 L amber glass solvent bottles that had been previously rinsed with sequential washes of hexane, ethyl acetate, acetone and methanol, then 3x sample rinses just prior to sample collection. Additional samples were collected on site for dissolved organic carbon; GF/F filtered and acidified, and filters collected for particulate matter analysis. All water samples collected were stored on ice for transport to the lab, where they were filtered through combusted GF/F filters prior to solid phase

extraction (SPE). Filters were collected for separate extraction. As PAEs are ubiquitous lab contaminants (24), all filtrations and extractions were performed in an organic clean-room, all glassware was thoroughly solvent rinsed and baked at 450 °C prior to use, and all Teflon® (solvent bottles, sample delivery system) thoroughly solvent rinsed. No other plastic was utilized in sample collection or extraction, in order to minimize contamination. All samples were stored at 4 °C, then filtered within 48 hours of collection, and extracted within 48 hours of filtration.

Table 5.3: Spring sampling; collection dates and water quality parameters. n/a=not available.

Date	WWTP/ Flow (MGD)	Flow (MGD)	Sample type	Final effluent temp °C	pH	Dissolved organic carbon (mg/L)
3/23/12	Ansonia	1.8	24h composite	17.2	6.7	6.96
3/29/12	Bridgeport Westside	21.5	Grab	14.7	6.9	5.00
3/2/12	Derby	1.8	24h comp + grab	11.7	6.7	5.77
5/4/12	Fairfield	8.7	24h composite	15.6	6.8	4.95
5/4/12	Greenwich	9.8	24h composite	16.7	6.8	6.00
5/4/12	Mattabassett	16.9	24h composite	18.3	6.8	n/a
3/2/12	Milford Housatonic	6.1	24h comp + grab	14.0	6.2	4.55
5/4/12	New London	6.7	Grab (n=2)	16.1	6.7	5.00
3/29/12	Shelton	2.19	24h composite	14.4	6.8	4.14
5/4/12	Stamford	16.8	24h composite	18.1	7.0	5.78
3/23/12	Stratford	6.3	24h composite	18.2	6.7	6.36

Table 5.4: Summer sampling collection dates and water quality parameters. (DOC measurements were not obtained due to analytical error). (n/a = not available).

WWTP/	Dates	Flow (MGD)	Sample type	Final effluent temp °C	pH
Ansonia	7/17/12	1.3	24h composite	23.3	6.8
Derby	7/17/12	1.4	24h composite	21.1	6.8
Milford Housatonic	7/17/12	4.9	24h composite	24.2	6.7
Shelton	7/17/12	1.9	24h composite	23.3	6.4
Stratford	7/17/12	6.0	24h composite	25.0	6.9
Milford Beaverbrook	7/17/12	1.5	24h composite	22.5	6.7

5.3.2 Experimental

Solid-Phase Extraction: PAE standards were purchased from Accustandard; dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butylbenzyl phthalate (BBP), di-ethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP), (d4) dibenzyl phthalate was used as recovery standard for extractions. All solvents used were obtained from Fisher, and were either Optima or pesticide grade. C18 resin was obtained from UCT Clean-Up extraction columns. Empty 6 mL glass solid phase extraction (SPE) cartridges were thoroughly cleaned and triple solvent rinsed before addition of 0.5 g of C18 sandwiched between two PTFE frits. C18 was pre-cleaned by sequential washes of 6 mL hexane, ethyl acetate, methanol, then conditioned with 5mL of ultrapure water. 1 L of filtered final effluent samples or 2 L of river or estuary water were passed through the SPE columns at a flow rate of approximately 2 drops per second. Sample containers were washed with 10% methanol, the rinse also passed through the SPE columns then the SPE columns were dried under vacuum for 30 mins. Samples were eluted with 6 mL of ethyl acetate, and elutes reduced to 0.5 mL under a

gentle stream of nitrogen, and transferred to a GC vial. Benzyl benzoate (500 ng) was added as an internal standard.

Table 5.5: Sampling station information and collection parameters for the summer low flow survey along the Housatonic River HR and estuary (nm=not measured).

Station	Location	Date	Salinity (psu)	Water temp °C	pH	Dissolved organic carbon (µg/L)
HR 1	41°09'35" N 73°06'05" W	7/24/12	27.53/ 27.59	22.66	7.94	1100.9
HR 2	41°10'23" N 73°06'48" W	7/24/12	27.49/ 27.53	22.77	7.76	1728.1
HR4	41°12'12" N 73°06'35" W	7/24/12	22.92	23.33	7.60	2549.5
HR 8	41°17'17" N 73°04'13" W	7/24/12	0.35	25.73	nm	nm
HR 9	41°18'53" N 73°05'08" W	7/24/12	0.13	26.20	nm	nm
HR4- day 2	41°09'35" N 73°06'05" W	7/25/12	7.991	24.65	7.48	1704.9
LIS1	41°09'35" N 73°06'05" W	7/25/12	20.02	nm	7.48	2778.5
LIS 2	41°07'42" N 73°02'56" W	7/25/12	27.49	22.34	7.98	2059.5
LIS 3	41°10'29" N 73°04'42" W	7/25/12	27.66	21.51	7.70	1838.2
LIS 4	41°08'40" N 73°06'23" W	7/25/12	26.68	21.78	7.60	912.9
LIS 5	41°08'25" N 73°08'01" W	7/25/12	27.09	nm	7.66	1468.8
LIS 6	41°06'42" N 73°07'54" W	7/25/12	27.20	nm	7.67	2990.5
LIS 8	41°06'33" N 73°12'34" W	7/25/12	27.27	nm	7.65	2069.1

GFF filter extraction: Following sample filtration, the GF/F filters were collated into cleaned glass vials with PTFE screw cap lids, frozen, and then lyophilized overnight. Filters were extracted in ethyl acetate (EA) approximately 10 mL to cover, and then stored at 4 °C overnight. Samples were sonicated for 60 mins, and then the EA was poured into a second glass vial. The extraction procedure was repeated once more, both elutes were collated and reduced to 0.5 mL under a gentle stream of ultra-high purity nitrogen gas. All samples were 0.2 µm filtered (PTFE) prior to GC-MS analysis.

GC/MS Analysis: A DB-5ms capillary column (30m x 0.25mm x 0.25µm) was used. The GC injector port temperature set at 250 °C; transfer line set at 270 °C; and electron impact (EI) ion source was set at 280 °C. Ionization energy was 70eV. The GC oven temperature program was as follows: 100 °C hold (1 min); 10 °C/min to 200 °C, then 15 °C/min to 260 °C, followed by 3 °C/min to 300 °C, hold (2 min). Carrier gas (ultra-high purity helium) was at constant flow of 1 mL/min. A 5 minute solvent delay time was used. Ions monitored in selective ion mode (SIM) are given in Table 5.6. Dibenzyl phthalate- 3,4,5,6-d₄ (DBP-d₄) (100 ng absolute mass) was spiked into each 0.5 L sample (n=3 filtered, n=3 unfiltered) prior to extraction for use as a recovery standard. Butyl benzoate (500 ng/ml) was added as an internal standard. Instrument detection limits (IDL) were determined by linear regression from the lowest concentration value by extrapolation from the calibration to where the peak to peak signal to noise ratio is equal to 3 (the greater value is given) (Table 5.7).

Table 5.6: GC/MS SIM mode parameters.

SIM: Fragment (<i>m/z</i>)		
PAE	Quant (Qual)	t_r (min)
DMP	163 (194, 77)	7.30
DEP	149 (177, 121)	9.03
DnBP	149 (223, 205)	12.82
BBP	149 (91, 206)	15.58
DEHP	149 (167, 279)	16.93
DnOP	149 (279, 167)	18.57
DBP-d4	153 (91, 107)	19.00
BB	105 (212, 91)	11.02

Table 5.7: Instrument detection limits (IDL) and peak-peak signal to noise ratio (S/N) of lowest calibration standards detected (SIM)

PAE	IDL (ng/mL)	Calibration standard (ng/mL)	Peak-peak S/N
DMP	6.4	1	9.9
DEP	6.5	1	5.4
DnBP	11.1	1	7.9
BBP	10.4	10	9.0
DEHP	9.4	10	6.5
DnOP	11.5	10	3.2

5.3.3 QA/QC

For the initial SPE extraction method development, 1 L amber glass bottles were filled with MilliQ water and spiked with 100 ng of each of the six target PAEs. Recoveries for DMP, DEP, DnBP, BBP and DnOP were between 95% - 112% however recoveries of DEHP were over 300%, (0.34 – 0.49 µg L⁻¹) which indicated the

possibility of DEHP contamination in the MilliQ water obtained from the point of use (POU) dispenser. The presence of DEHP contamination in Ultra High Purity water has in fact been previously reported by Liu *et al.*, where, in their 2008 paper, the authors report the presence of DEHP in the water passing through a reverse osmosis (RO) filtration system, in concentrations ranging from $1.89 \mu\text{g L}^{-1}$ (at the RO permeate stage) to $0.20 \mu\text{g L}^{-1}$ at the POU, consistent with the concentration of DEHP detected from the MilliQ POU water in this study (34).

During the field sampling campaigns, 1 L amber glass bottles containing a small volume (100 mL) of MilliQ water served as field blanks. Process blanks for SPE and GFF extractions were carried out with each sample batch. Background contamination with phthalates is common in laboratories, where contamination may occur through exposure to dust and plastic laboratory products (24). Despite all efforts to reduce PAE contamination, all 6 target PAEs were detected in the SPE and GFF extraction blanks and field blanks. However, the background concentrations were generally consistent, therefore the process blanks were used to correct for the field samples by setting the limit of quantitation (LOQ) as equal to the highest blank concentration measured, plus 3 standard deviations of the blanks (Table 5.8 A-C). The PAE blank concentrations observed in this study were also consistent with those reported by Xie *et al.*; who reported mean blank concentrations of 9.7, 10.2, 3.7, 0 and 43.7 ng/L for DMP, DEP, DnBP, BBP and DEHP respectively (7). Field sample data were blank corrected by subtracting the largest measured PAE concentration in the process or field blanks.

Recovery of the surrogate standard d_4 -dibenzylphthalate was within acceptable ranges for each sample set: for the WWTP effluent SPE, recoveries range from 65% -

104% (n = 6, mean = 81%, standard deviation = 14.6%), for the river and LIS water samples recoveries ranged from 61% - 136% (n= 17, mean = 97%, standard deviation = 23.3%).

Table 5.8 A-C: PAEs in process blanks and LOD parameters.

A: WWTP survey- aqueous phase (SPE) extractions; contamination concentrations (ng/mL; in extracts with final sample volume of 1 mL). (nd =not detected.; nd* = not detected in blanks or samples). While SPE background signals of DnBP were generally consistent, GFF extraction data was erratic indicating possible contamination, therefore all DnBP data was excluded. Limit of detection (LOD) = highest concentration measured in blank plus 3 x standard deviation of blanks.

PAE	SPE blank 1	SPE Blank 2	Field Blank 1	Field Blank 2	Standard deviation	LOQ
DMP	6.85	6.86	8.98	9.49	1.39	13.7
DEP	7.16	7.73	9.99	8.85	1.25	13.8
BBP	13.11	13.67	nd	nd	0.4	14.9
DEHP	41.06	32.06	34.56	33.46	3.98	53.0
DnOP	12.19	12.07	11.98	12.82	0.38	14.0

B: WWTP survey- Filter/particulate phase extractions (**Use DMP standard deviation to derive LOQ):

PAE	GFF extraction blank 1	GFF extraction blank 2	Standard deviation	LOQ
DMP	11.1	9.8	0.92	13.9
DEP	10.9	nd	**	13.7
BBP	nd*	nd*		nd*
DEHP	57.5	61.7	2.97	70.6
DnOP	11.7	11.9	0.13	12.3

C: Housatonic WWTPs, river and estuary survey, July 17th: aqueous phase SPE extraction contamination concentrations (ng/mL).

PAE	Field blank 1	Field blank 2	Field blank 3	Standard deviation	LOQ
DMP	nd*	2.0	2.3	0.21	2.9
DEP	2.5	5.5	9.0	3.25	18.9
BBP	18.0	46.9	38.8	14.91	64.6
DEHP	44.2	47.0	37.1	5.1	62.2
DnOP	nd*	nd*	nd*		nd*

5.4 Results and discussion

5.4.1 Survey 1: Concentrations of PAEs in the final effluent samples from 11 CT WWTPs

With the known high potential for laboratory contamination of PAEs due to their ubiquitous presence in the laboratory environment, the PAE concentration data obtained were compared to those reported in the literature, specifically the relative frequency in which each PAE is mostly commonly detected. DEHP is generally the predominant PAE in wastewaters, accounting for approximately 50% of the PAE mass, with the remaining 50% distributed between the PAEs in the following mass order: DEP > DMP > DnOP > BBP (35). The results from the CT WWTP survey conducted in both spring and summer were consistent with this literature trend in congener ratios.

Derby and Milford Housatonic were the first WWTPs sampled in March of 2012. Composite samples were collected for duplicate extractions using two different volumes (0.5 L and 1 L), plus one additional grab sample (0.5 L), in order to evaluate sample volume on PAE extraction efficiencies, and compare the PAE concentrations obtained by using either composite and grab sampling techniques. Results from this initial screening survey are given in Table 5.9, (concentrations given have been blank corrected by subtracting the highest concentration detected in either the field or SPE reagent blanks – see Table 5.8). The results showed that the larger volume (1 L compared to 0.5 L) SPE extraction performed slightly better for recoveries of PAEs present in lower concentrations. Therefore for the remaining WWTPs surveyed, 1 L samples were obtained. Overall the data for the PAEs measured in the aqueous phase from grab samples was comparable to that in the composite samples.

Table 5.9: Concentrations (ng/L) of target PAEs in 1L and 0.5L samples of WWTP final effluent. Values are blank corrected. (aq) = aqueous phase. (SPM) = suspended particulate matter phase (retained on the GF/F filter- given in units of ng PAE/L filtered effluent); nd= not detected.

WWTP	Sample	DMP	DEP	BBP	DEHP	DnOP
Derby	composite 1L (aq)	4.0	5.0	<LOQ	6.8	nd
	composite 0.5 L (aq)	<LOQ	6.0	nd	Nd	nd
	grab sample 0.5 L (aq)	<LOQ	11.8	nd	25.7	nd
Derby	composite 0.825 L (SPM)	<LOQ	9.5	nd	32.5	nd
	composite 0.855 L (SPM)	<LOQ	5.0	nd	84.8	0.3
	grab 0.92 L (SPM)	<LOQ	9.8	nd	79.6	nd
Milford Housatonic	Composite1L (aq)	<LOQ	5.9	<LOQ	18.3	nd
	composite 0.5L (aq)	<LOQ	12.7	nd	<LOQ	nd
	grab 0.5 L (aq)	<LOQ	6.3	nd	<LOQ	nd
Milford Housatonic	composite 1 L (SPM)	<LOQ	7.7	nd	200.3	2.0
	composite 0.83 L (SPM)	<LOQ	10.0	nd	142.0	2.2
	grab 0.96 L (SPM)	<LOQ	10.8	nd	30.9	nd
New London	grab 1 L (aq)	nd	3.4	nd	<LOQ	nd
	grab 1 L (aq)	nd	4.0	nd	<LOQ	nd

Table 5.10: Concentrations (ng/L) of PAEs (aq + SPM phases) detected in WWTP final effluent samples, collected spring 2012. Concentrations have been blank corrected. Nd= not detected. *Detected in SPM phase only.

WWTP	DMP	DEP	BBP	DEHP	DnOP
Ansonia (n=1)	<LOQ	8.0	nd	587.3	8.6
Bridgeport Westside (n=1)	<LOQ	5.9	nd	<LOQ	nd
Derby (n=3)	<LOQ – 4.0	11.0– 21.6	<LOQ	39.9 – 105.3	nd - 0.3
Fairfield (n=1)	<LOQ	3.2	<LOQ	60.3	0.3
Greenwich (n=1)	<LOQ	9.8	<LOQ	60.4	0.3
Mattabasset (n=1)	2.8	41.8	nd	123	3.3
Milford Housatonic (n=3)	<LOQ	13.6 –22.8	nd	30.9 – 218.6	nd – 2.1
New London (n=2)	<LOQ	3.4 – 4.0	nd	87.2*	nd
Shelton (n=1)	2.3	35.5	nd	227.2	nd
Stamford (n=1)	<LOQ	5.4	nd	64.2	nd
Stratford (n=1)	nd	8.1	nd	50.6	13.4

For the 11 CT WWTPs surveyed, DEHP and DEP were the most predominant PAEs detected (Table 5.10), present in 100% of the samples with the exception of DEHP in Bridgeport which was not detected above the LOQ following blank correction (see Table 5.8 for LOQ and blank DEHP values). The average total (sum of aqueous and particulate phases) PAE concentrations measured, and total mass flux of PAEs (mg/day, calculated from measured concentrations x daily flow rate of the WWTP) are presented in Figure 5.2. Average concentrations for this data set are 148.4 ng/L for DEHP, 13.6 ng/L for DEP, and 4.0 ng/L and 3.0 ng/L for DnOP and DMP respectively. Consistent with the literature, DEHP accounts for the majority of PAEs present in final

effluent, followed by DEP (20), however the concentrations detected for this survey are generally much lower than those reported in the literature, where concentrations of DEHP are commonly measured in the $\mu\text{g/L}$ range (Table 5.11). Ansonia was the only facility with measured concentrations of DEHP approaching the $\mu\text{g/L}$ range; this same facility was also observed to have one of the highest perfluoroalkyl acid (PFAA) concentrations measured in final effluent (Chapter 3, section 3.1). PAEs measured in WWTP effluent in Oakland, CA, by Jackson and Sutton in 2008 (24) were generally greater than those measured in this study, however the CT PAE measurements were within the concentration ranges reported for CA wastewater effluent (Table 5.11). Of important note in comparison to the data obtained in this study, the reported range of PAE concentrations observed in the three WWTPs surveyed in Oakland, CA by Jackson and Sutton were also highly variable, ranging up to three orders of magnitude. The maximum PAE concentration range between plants in this study was for DEHP, ranging from <LOQ to 587 ng/L. Highly variable PAE concentrations were also reported in WWTP effluents in Austria (28) and France (32).

The low effluent PAE occurrence in this study is likely a function of the spring WWTP seasonally higher flow rates and thus increased dilution factor. However, the lower concentrations may also be a reflection of the increased restriction in use of PAEs in Canada and the US, with regulatory limits set in 2007 on the concentration of larger molecular weight congeners such as DEHP and DnOP in childcare articles, and an overall sharp decline in total use reported from 2010 to 2012 (36).

Table 5.11: Literature values reported for PAE concentrations in WWTP final effluents (ng/L).

Values given are averages, averages (range) or average \pm standard deviation.

Location	DMP	DEP	DnBP	BBP	DEHP	DnOP	Ref. Year
Germany					1740- 182000		(29) 2002
USA		<LOQ-1000	570- 5500	<LOQ-740	210-1000		(24) 2008
France	80	780	150	300	5420	20	(37) 2009
Spain	130	49800		10	9430		(38) 2009
Austria	62 (<LOQ- 190)	200 (<LOQ- 1100)	540 (nd- 2400)	360 (90- 1400)	1600 (80 - 6600)	17 (<LOQ- 260)	(28) 2010
France	30 \pm 30	40 \pm 50	140 \pm 100	160 \pm 150	2000 \pm 1200	10 \pm 10	(32) 2014

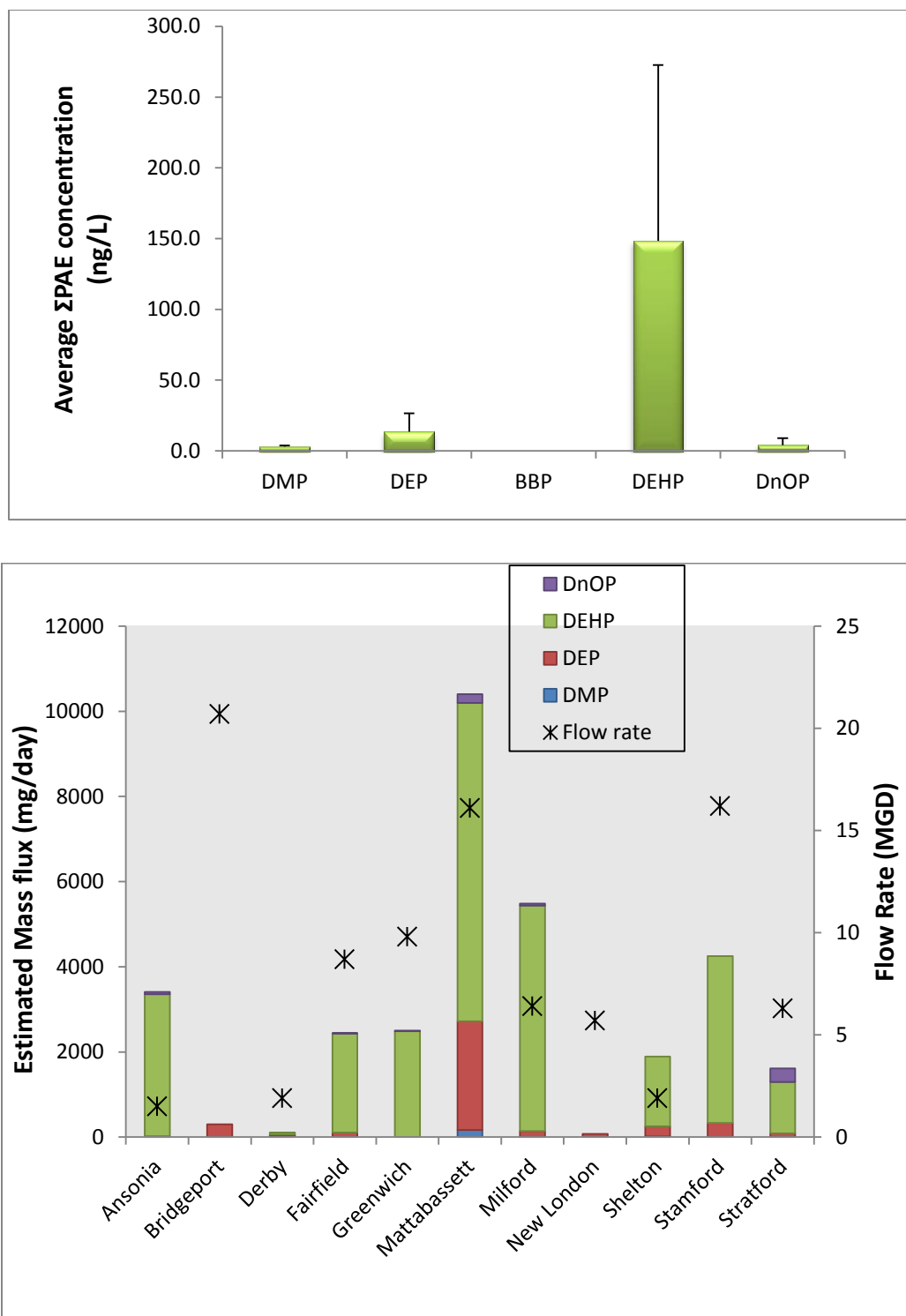


Figure 5.2: Average total (aqueous and SPM phase) PAE concentrations in final effluent (ng/L) (error bars are standard deviation) (top), and estimated total PAE mass flux (mg/day) from the 11 CT WWTPs surveyed spring 2012 (bottom).

DEP, DEHP and DnOP were found associated with the SPM phase in concentration ratios reflective of those detected in the aqueous phase. DEHP was the predominant PAE associated with SPM, detected in 100% of samples and above LOQ in 73%, with concentrations ranging from <LOQ to 237.5 ng/L of effluent filtered. The highest DEHP concentrations measured at Ansonia WWTP, consistent with data for the aqueous phase. DnOP was detected in 18% of aqueous phase samples, but in 64% of the SPM phase samples. DEHP was detected in 100% of aqueous and SPM samples, whereas DEP was measured in 91% of aqueous and 64% of SPM. On average, 44.9% of DEP and 74.6% of DEHP total concentrations were associated with the SPM phase. A similar relationship was reported by Teil *et al.* (33), where distribution between particulate and dissolved phases were reported to be 33.6% for DEP and 66.4% for DEHP.

The concentrations of PAEs associated with the WWTP effluent SPM (efSPM) and mass fluxes of particulate fraction PAEs, calculated from the efSPM concentration, the WWTP daily flow rate and the measured concentrations of PAEs on the particulate phase, are detailed in Table 5.12. Using the concentrations of DEP associated with the efSPM fraction of Ansonia WWTP effluent as an example, values were calculated as follows:

WWTP	Flow Rate (MGD)	[efSPM] mg/L effluent	[PAE]-efSPM
Ansonia	1.5 Mega gallons per day (MGD)	4.1 mg efSPM/L	3.9 ng DEP /L
$[PAE]_{efSPM} = \frac{[PAE] - efSPM}{[efSPM]}$		$[DEP]_{efSPM} = \frac{3.9 \frac{ng}{L}}{4 \frac{mg}{L}} = 0.94 \frac{ng}{mg}$ $= 0.9 \text{ mg DEP/kg efSPM}$	
SPM daily flux (kg/day): $efSPM_{d^{-1}} = \frac{efSPM (mg)}{L} \times \frac{3.79 \times 10^6 L}{MG} \times \frac{Flow rate (MG)}{Day}$		$= \frac{4.1 \text{ mg efSPM}}{L} \times \frac{3.79 \times 10^6 L}{MG} \times \frac{1.5 MG}{Day}$ $= 23.5 \text{ kg SPM day}^{-1}$	
Daily mass flux PAE assoc. /w efSPM: $[PAE]_{efSPM_{d^{-1}}} = [PAE]_{efSPM} \times efSPM_{d^{-1}}$		$= \frac{0.94 \text{ mg DEP}}{\text{kg efSPM}} \times \frac{23.5 \text{ kg efSPM}}{\text{day}}$ $= 22.1 \text{ mg DEP}_{efSPM} \text{ day}^{-1}$	

The concentration values for DEHP associated with the efSPM phase are greater than the predicted environmental risk limit for sediment concentrations (ERL_{sediment}) derived in the risk assessment of van Wezel (6) of 2.8 mg/kg (dry wt.) for endpoints related to endocrine disruption (39). Although the efSPM-DEHP concentration does not reflect the final sediment phase DEHP concentration in receiving waters due to the dilution of SPM with riverine and/or estuarine SPM in the discharge zone, the potential of high exposure to benthic organisms from the daily efSPM-DEHP mass flux may be of particular concern due to efSPM being regarded as a high quality food source that is preferentially assimilated by riverine benthos (40). Further research is warranted to investigate the potential impact of efSPM as a vector of DEHP to the food chain.

Table 5.12: Concentrations of DEP, DEHP and DnOP detected on the filters, given in μg per g of SPM, and daily mass flux ($\mu\text{g}/\text{day}$) calculated from the concentration of SPM x the flow rate of the WWTP. LOQ = limit of detection. Nd = not detected.

WWTP	[DEP] (mg/kg SPM)	Mass flux DEP assoc. w/efSPM (mg/day)	[DEHP] (mg/kg SPM)	Mass flux	[DnOP] (mg/kg SPM)	Mass flux
				DEHP assoc. w/efSPM (mg/day)		DnOP assoc. w/efSPM (mg/day)
Ansonia	0.9	22.1	57.3	1348.5	0.1	3.2
Bridgeport	1.0	164.5	<LOQ		Nd	
Derby	4.5	58.3	36.1	472.0	0.2	2.4
Fairfield	<LOQ		32.0	1643.2	0.2	10.0
Greenwich	5.8	363.5	29.9	1872.5	0.2	12.6
Mattabassett	<LOQ		32.2	6642.3	0.3	51.5
Milford	4.3	230.1	56.3	3013.4	1.0	50.9
New London	<LOQ		35.3	1880.6	Nd	
Shelton	0.7	15.8	59.8	1454.2	Nd	
Stamford	<LOQ		22.0	2980.0	Nd	
Stratford	1.1	107.3	<LOQ		3.3	318.3

A significant ($P < 0.01$) inverse relationship was observed between DOC and the % of DEHP associated in the particulate phase in final effluent (Figure 5.3), indicating DOC enhancement of the apparent solubility of DEHP due to partitioning to the colloidal fraction. DOC has been suggested to facilitate the transport of hydrophobic organic pollutants through the WWTP process and out into receiving waters (3). A correlation between DOC and the transport of DEHP from landfill sites in leachate waters was previously reported by Zhang *et al.* (41).

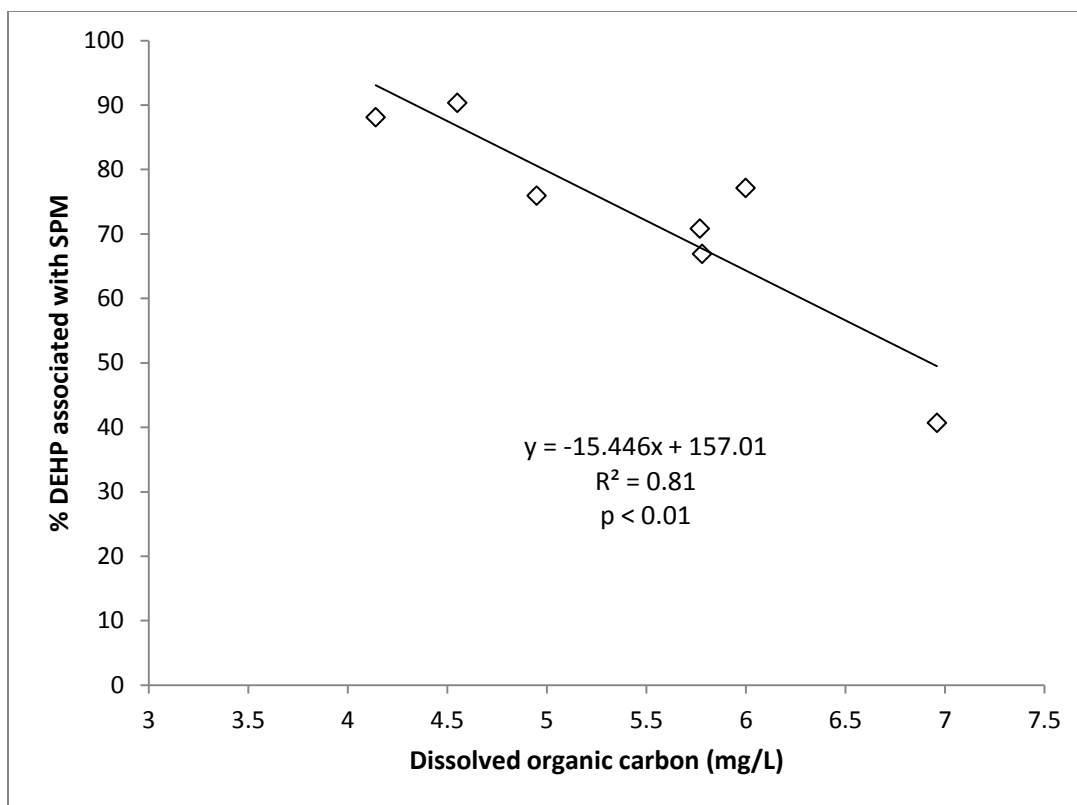


Figure 5.3: Inverse relationship between concentration of dissolved organic carbon (DOC) and the % of total DEHP associated with the SPM phase in effluent water samples.

Partitioning constants were derived for DEHP from the wastewater effluent aqueous and SPM phases. Organic carbon normalized constants were derived assuming an average effluent SPM organic carbon content of 30%, consistent with that observed for the wastewater survey reported in Chapter 3. As observed in Chapter 3 for the longer chain PFAA congeners, the partitioning constants derived were greater than this previously published. The log K_{oc} value obtained for DEHP of 6.76 ± 0.43 for efSPM partitioning was found to be at least an order of magnitude greater than the range of log K_{oc} values published in the peer reviewed website within the US National

Library of Medicine, the Hazardous Substances Data Bank (HSDB) (42), of between 4.94 – 5.71. In regard to the relationship between DOC and %DEHP associated with the SPM phase described in Figure 5.3, lower partitioning coefficients would have been expected within effluent streams due to increased apparent solubility as a function of the presence of DOC. This would result in lowering the ratio between the solid and aqueous fractions. However, the derived Koc value does not negate the potential for DOC to enhance DEHP solubility, as the additional factor of low particulate concentrations has been shown to have a key effect on partitioning coefficients derived for DEHP with SPM. Turner and Rawling in a 2000 study found an inverse relationship to exist between the partition coefficients derived for DEHP and the concentration of riverine and estuarine particulates (22). The authors report a regression equation; $K_d = 2.63 \times 10^6 \cdot \text{SPM}^{-1.15}$ describing the linear relationship between the log K_d and the log riverine SPM concentrations values.

While the range in particulate concentrations observed in the WWTP effluent samples were not as great as the range of SPM concentrations used in the study by Turner and Rawling, a similar relationship was observed in this study, though with limited data points and therefore low significance, as depicted in Figure 5.4. However, a similar linear relationship expression was derived for this data set; $K_d = 8 \times 10^6 \cdot \text{SPM}^{-1.75}$. Both DOC and SPM concentration therefore seem to be affecting the distribution behavior of DEHP in the final effluent waters; while DOC appears to enhance DEHP solubility, the partitioning coefficients derived also suggest that efSPM bound DEHP will be of greater concentrations than may be predicted using the Koc values published by the National Institute of Health (42) due to the low

suspended solid concentration. These observations are clearly speculative with a limited data set, and a controlled laboratory experiment to investigate efSPM-DEHP partitioning is warranted, as well as to determine the extent of the potential filter artifact which is likely affecting these preliminary partitioning coefficients derived.

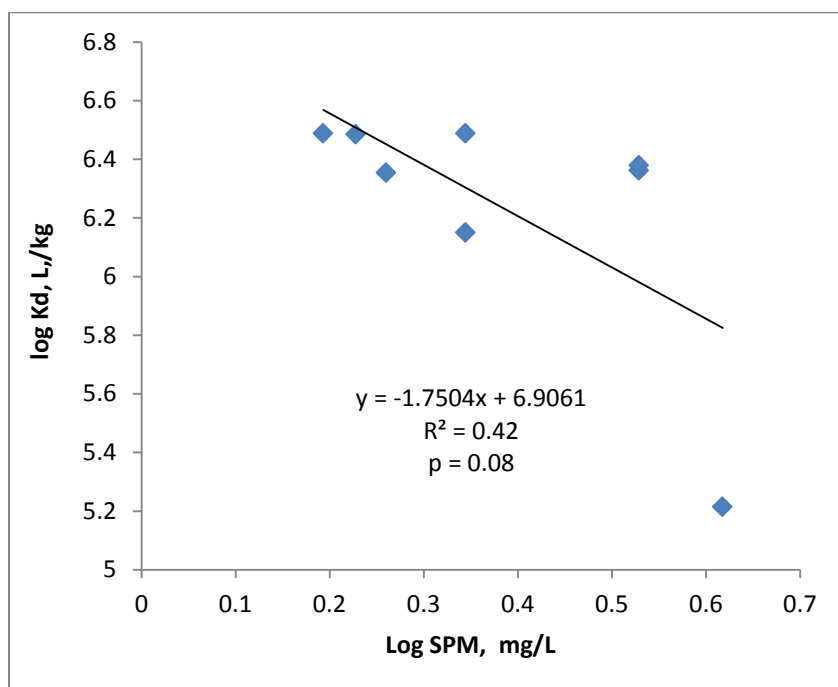


Figure 5.4: Distribution coefficients (as log Kd) for DEHP adsorption to effluent SPM, as a function of effluent particulate concentration.

5.4.2 Survey 2: Concentrations of PAEs from 6 CT WWTPs and in the receiving waters of the Housatonic River and LIS estuary.

PAEs were measured in the final effluent samples obtained from 6 WWTPs located along the lower Housatonic River estuary, July 17, 2012. The following WWTPs had been previously sampled in the spring; Ansonia, Derby, Shelton, Milford

Housatonic, and Stratford. An additional treatment plant, Milford Beaverbrook, located near the mouth of the Housatonic River, was also sampled. Effluent waters were processed as previously, with filtration preceding extraction of both suspended particulate and aqueous phases. LOQs for the aqueous phase extractions were comparable to those obtained in the previous WWTP survey (Table 5.8). Unfortunately the GF/F extraction blanks were found to have high levels of contamination of all PAEs; therefore the data presented for the July survey is for the aqueous phase only.

In WWTP final effluent, DEHP was again the predominant PAE, however the concentrations measured were on average 4x higher for DEHP in the summer and detected in 100% of samples above the LOQ compared to 83% in spring. DEP was detected in 83% of samples, up from 67%, however there was no increase in average concentration. Data for the PAEs detected in the aqueous phase from WWTP effluents is presented in Table 5.13, and compared in Figure 5.5.

DEHP in effluent from Ansonia was measured at a concentration (5575 ng/L) greater than the highest concentration calibration standard; therefore this value is reported as >1000 ng/L. With PAE data, contamination concerns lead the suspicions regarding large value measurements, however the consistency of the larger DEHP concentrations measured in both spring and summer 2012 at the Ansonia treatment facility corroborates the data obtained (Figure 5.5). Generally lower PAE concentrations were measured in the final effluents collected in the spring compared to the summer, which is likely attributable to a dilution factor since the WWTP flow rates were an average of 20% greater during the spring survey. The increased DEHP concentrations in the dissolved fraction of the summer effluent waters may also have been facilitated by

higher DOC concentrations with warmer temperatures, enhancing aqueous phase DEHP concentrations. While DOC measurements from the WWTPs sampled in the summer were not obtained, a 1.6x increase in final effluent DOC in summer compared to spring was reported by Vieno *et al.* (43).

Daily mass flux of DEHP (aqueous phase only) from the six WWTPs in the lower Housatonic River surveyed were calculated from flow rate: Ansonia- 4.9 g/day (considering the concentration to be 1000 ng/L, the highest calibration standard, or 27.4 g/day for 5575 ng/L actual concentration value obtained); Milford Housatonic- 8.4 g/day; Stratford- 2.7 g/day; Milford Beaverbrook- 1.1 g/day; Shelton- 1 g/day and Derby 0.2 g/day. The total DEHP daily mass flux to the HR from these 6 WWTPs was in the order of 18 g/day. Comparing the 5 WWTPs surveyed both spring and summer (dissolved phase only), the DEHP daily mass flux increased approximately threefold, from 6 g/day in the spring to 17 g/day (or 40 g/day if considering Ansonia measured DEHP concentration of 5575 ng/L) in the summer.

The impact of PAEs discharging from WWTPs into the HR and estuary was investigated July 23rd and 24th 2012. Surface water samples were obtained from sampling stations located along the length of the HR, from the mouth (HR 1) with stations numbered away from the mouth towards the fresh up river water (HR 9). LIS surface waters in the proximity of the HR mouth were also sampled: details on the locations of the HR and LIS sampling sites are given in Table 5.5. Concentrations of PAEs detected in the HR surface waters were 6 x less for DEHP and 3 x less for DEP than in the WWTP effluents; therefore WWTP discharge is a source of PAE contamination to the Housatonic River (Figure 5.6).

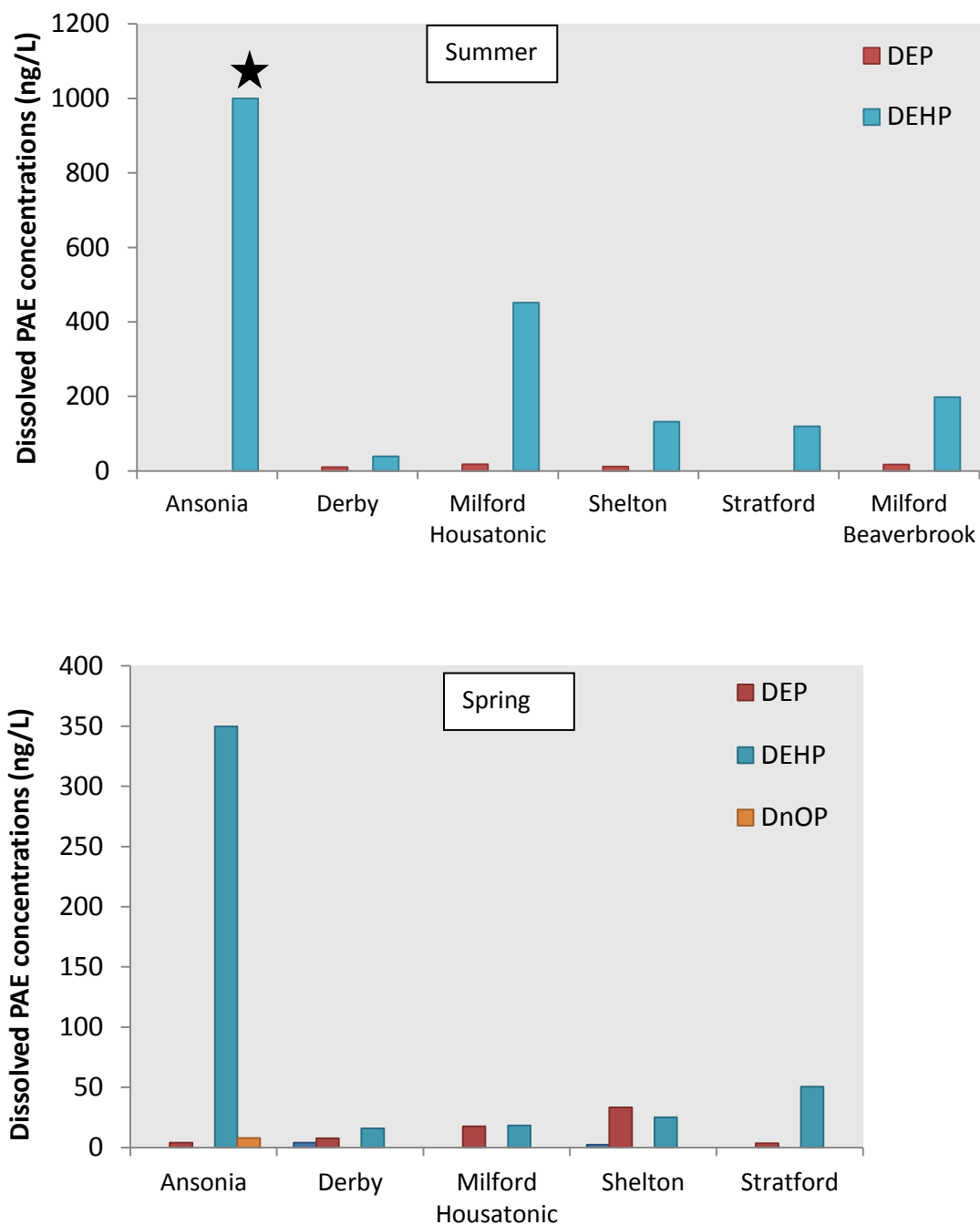


Figure 5.5: Top- Concentrations of PAEs (ng/L) detected in the aqueous phase only of final effluent from the 6 WWTPs located along the lower Housatonic River (HR), sampled summer 2012. Bottom- PAE aqueous phase concentrations (ng/L) measured in the 5 of the same 6 WWTPs located along the HR, sampled in spring 2012, shown for comparison. (★Measured concentration was > than the highest calibration standard, 1000 ng/mL).

Table 5.13: Aqueous concentration of DEP and DEHP measured in final effluent samples obtained from the six WWTPs located along the Housatonic River, July 2012.

WWTP	Concentration of phthalates detected in aqueous phase (ng/L)	
	DEP	DEHP
Ansonia	Nd	>1000
Derby	9.8	39
Milford- Housatonic	17.8	451.6
Shelton	11.7	132.5
Stratford	Nd	119.4
Milford- Beaverbrook	17.5	197.9

Unlike the distributions of PFAAs along the HR (Chapter 4), there was no clear evidence of a higher up-river to lower-down river concentration gradients, corresponding to the change in salinity with mixing river and estuarine waters, unlike the conservative mixing behavior described for the PFAAs detected in the Housatonic River as discussed in Chapter 4. PAE concentrations were observed to be lower at both the most up river site (HR 9) and at the river mouth (HR 1) on day 1 of the river survey. Highest PAE concentrations were measured at HR 4, which was located 2200 m downstream of the Milford Housatonic WWTP discharge zone. Conservative mixing behavior along the river is not an expected fate for longer chain PAEs; DEHP has a hydrophobic nature and with a K_d value > 10,000 is expected to interact strongly with particulates, an effect with increases with salinity (22, 23). The relatively higher DEHP concentrations measured in the dissolved phase of the saline waters at river station 4 may be indicative of a local source of DEHP at this location. Clara *et al.* (2010) reported high levels of

DEHP in runoff from traffic roads (28). At either side of the Housatonic River at this location are boat yards/marinas with parking areas, which are busy traffic areas in the summer, and therefore have the potential to be a source of surface runoff due to the rain shower the previous day. The PAE along river profile obtained for this field survey represents only a single snapshot in time, and further field work is required to confirm the presence of a local source resulting in a greater loading of PAEs to river near station 4, and to further assess the contributions from both point and non-point sources of PAEs to the waterway.

DMP, DEP and DEHP were detected in 100% of LIS surface water samples. Average DEHP concentrations were 4.4x higher in the LIS surface waters than those measured in the HR surface. DEP concentrations were 5.3x higher in LIS than HR. The highest concentrations of DMP and DEP were measured at LIS site 4. Surface DEHP concentrations measured on day 2 were observed to increase along the southward path of the HR, from HR 4 to LIS 1. LIS 2 and 3, on the south-eastern side of the river plume, and LIS 8 to the west had the highest DEHP concentration (Figure 5.7). Estuaries are reported to be a trap for phthalates such as DEHP. Reported estuarine model simulations incorporating the effects of salinity, particulate concentrations and biodegradations rates, indicate that up to 50% of DEHP discharged into a catchment may be retained by estuarine sediment over a timescale equal to the particle residence time (22). Data for the Housatonic River mouth may reflect the retention of DEHP within the estuary region, however increasing concentrations of DEHP away from the shoreline may also indicate that the Housatonic River and shoreline WWTPs are not likely to be the only important sources of PAEs to this region of the LIS. An increase in

PAE concentrations from upstream to downstream was also reported for the river Seine basin, with the impact of surface run-off determined to be greater than that of WWTP discharge (33). Phthalates released directly to the atmosphere are also reported to be an important source to the watershed from both wet and dry deposition (4). DnBP and DEHP have been reported to be the predominant PAEs in the atmosphere above an urban location, and detected in rain water in average concentrations of 600 ng/L and 400 ng/L respectively (44).

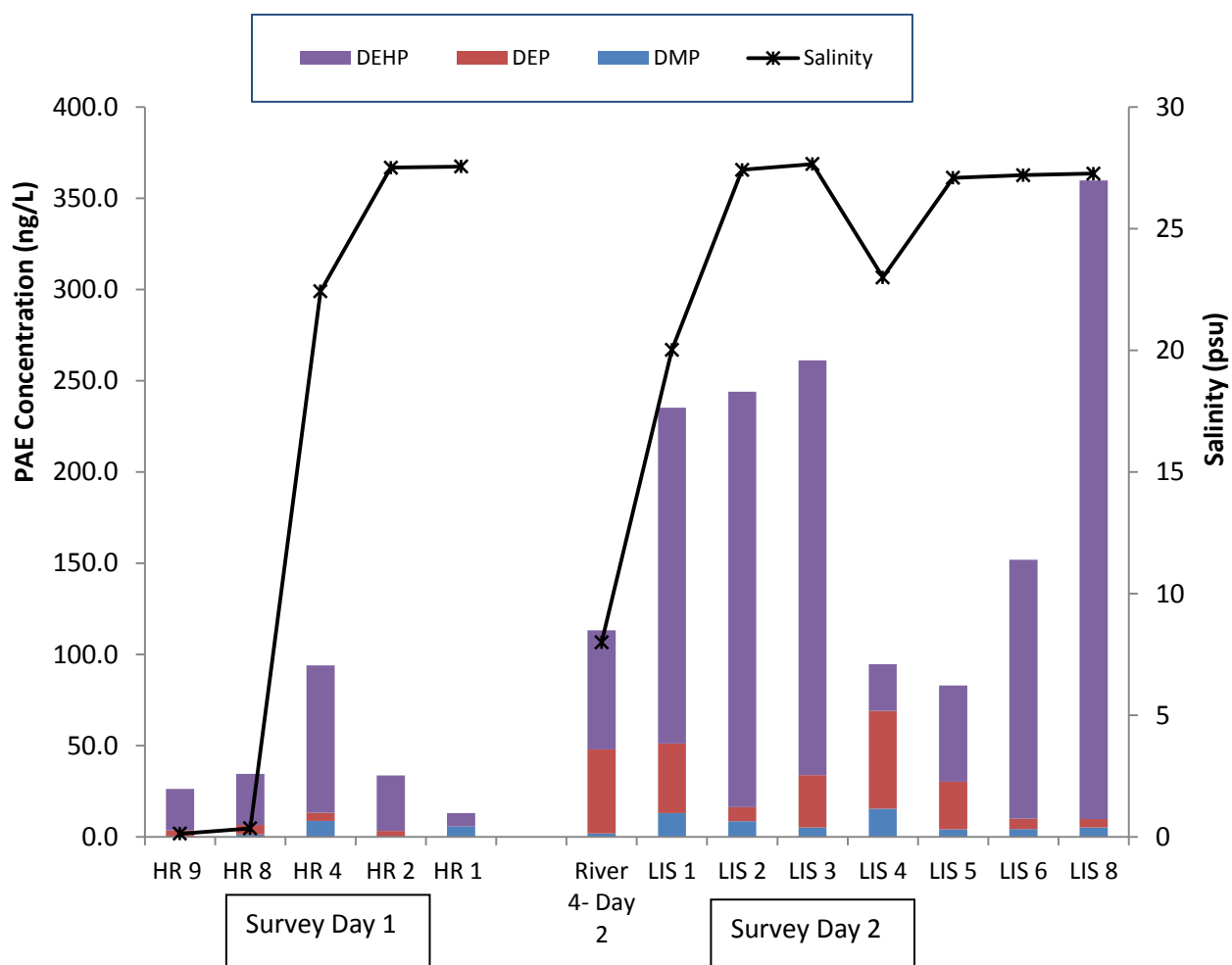


Figure 5.6: Concentrations of PAEs (ng/L) detected in the aqueous phase of surface waters sampled of the Housatonic River and estuary, July 2012.

The average concentration of DEHP in the shoreline LIS surface waters in this field study was 160 ng/L (26 – 350 ng/L min – max). The DEHP concentrations detected in the Housatonic region of the LIS shoreline surface waters were within the ranges of reported concentrations of DEHP in estuaries in the Netherlands, in the UK, and in Mississippi and Texas, although the maximum reported concentrations in all but the estuary in Mississippi were at least twice that seen in this study (Table 5.14). Riverine concentrations were also far lower in comparison to values reported for rivers in Germany, the Netherlands and the UK, and again it is highly probable that the lower PAEs measured in this region reflect the relatively recent actions taken by governmental agencies to manage PAEs in consumer products (Consumer Products and Safety Commission) food and food contact substances, cosmetics, pharmaceuticals and medical devices (Food and Drug Administration) (45)

However, the measured DEHP concentrations in the surface waters of the estuary were, in 4 of the 8 estuary stations, greater than the environmental risk limit (ERL) derived by van Wezel *et al.* of 190 ng/L. Van Wezel *et al.* derived an ERL for DEHP by considering endocrine disruptive effects data (6). Chikae *et al.* reported clear endocrine disruptive effects of DEHP on the Japanese medaka, *Oryzias latipes*, including delayed hatching, biased sex-ratios and the gonadosomatic index of males reduced in all the DEHP treated groups, but the pronounced effects were reported at the lowest aqueous DEHP concentrations of 10 ng/L and 100 ng/L (46, 47). This is a common feature of endocrine active compounds as higher concentrations lead to saturation of receptor binding sites, therefore effects do not follow a typical dose-response relationship.

In addition to the potential for endocrine disruption, DEHP has considerable bioconcentration potential, with bioconcentration factors (BCFs) reported in the ranges of 114 – 1380 in fish, and up to 5380 for filtrating mollusks (39). Average water phase DEHP (160 ng/L) could potentially therefore result in DEHP concentrations, in estuary fish species, of between 18 – 220 mg/kg. While DEHP does not appear to undergo biomagnification through a food web, as illustrated by the study by Mackintosh *et al.*, (48) where DEHP, in contrast to PCBs, did not exhibit significant trends with trophic levels determined using stable nitrogen isotopes, there is still however the concern regarding the potential risk from secondary poisoning due to high DEHP levels in the seafood predominantly eaten by predatory animals, including humans.

Further investigations into the potential role of suspended particulate matter in the fate and transport of PAEs in both fresh river and saline estuary waters, were unfortunately hampered due to compromised filtration blanks. However, the amount of DEHP measured in the aqueous phase samples of surface waters did show a positive linear relationship with the concentration of dissolved organic carbon in both riverine and estuarine samples (Figure 5.8), mirroring the trend observed in the WWTP effluents that indicated a decreased partitioning to SPM with increasing DOC. A similar trend was observed by Teil *et al.* (33) for aqueous DEP and DOC in river water samples.

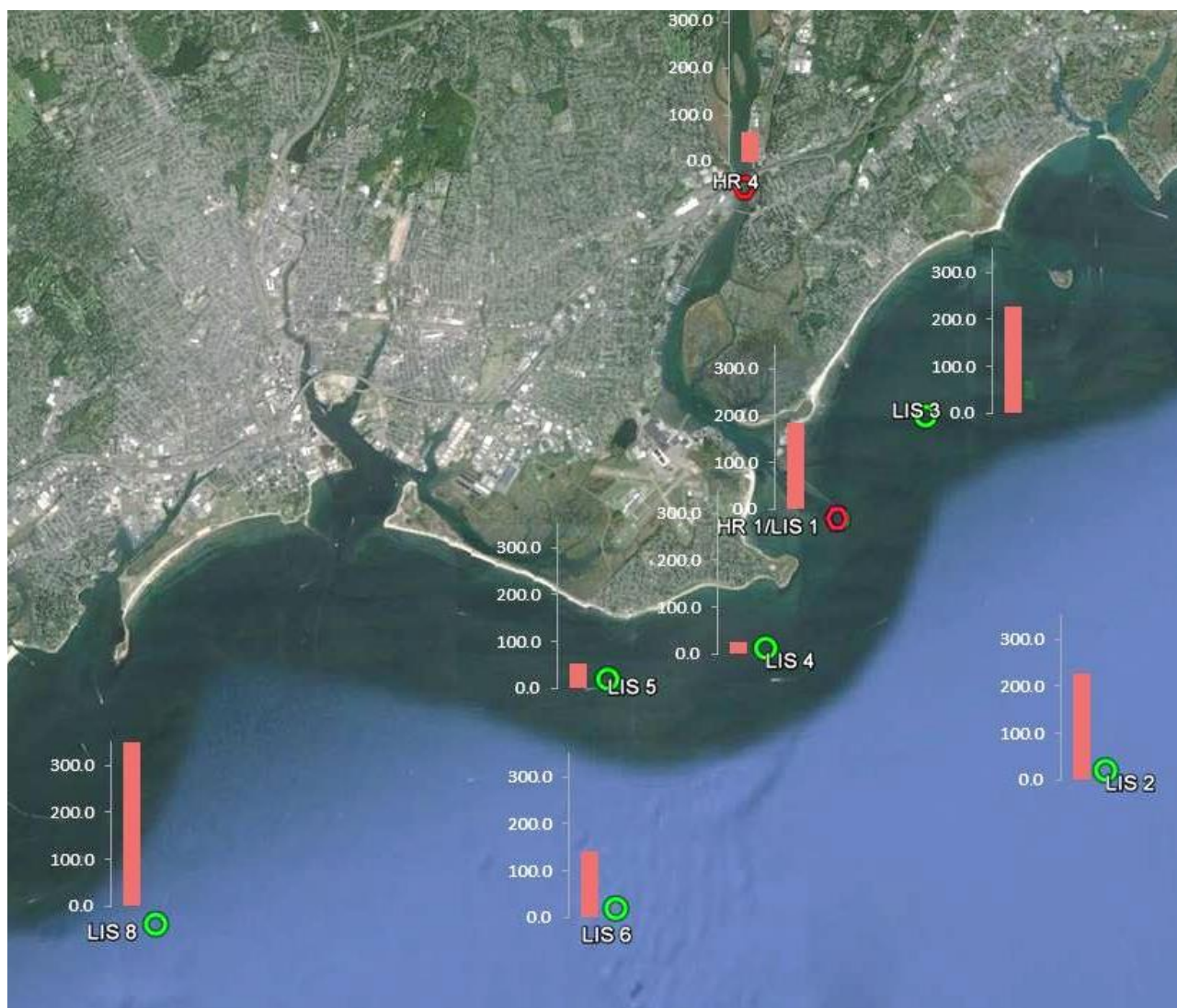


Figure 5.7: Surface water concentrations of aqueous phase DEHP (ng/L) in the coastal LIS, measured in samples obtained July 25th, 2012 (survey day 2).

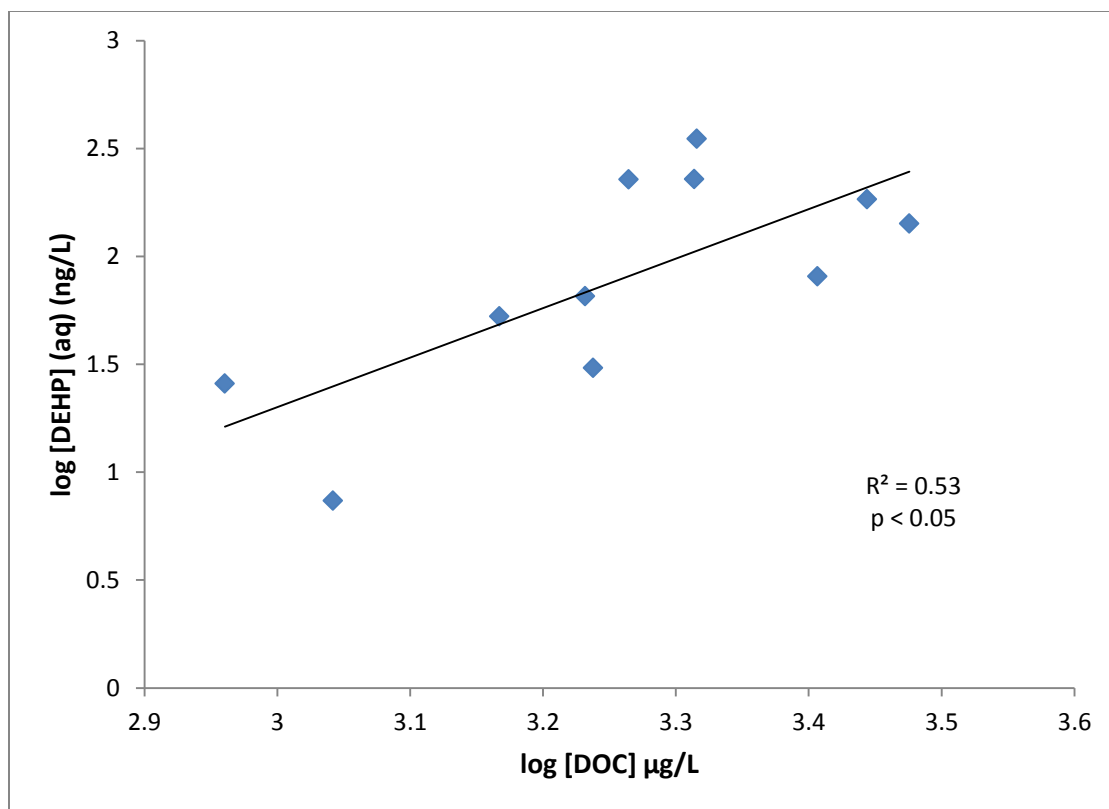


Figure 5.8: Relationship between concentrations of DEHP in apparent dissolved phase, and concentrations of dissolved organic carbon in HR and LIS surface water samples.

Table 5.14: Literature values reported for PAE concentrations in rivers and estuaries. Values given are averages, averages (range) or average \pm standard deviation (ng/L).

Location, sample	DMP	DEP	DnBP	BBP	DEHP	DnOP	Ref. Year
Germany, river			12-8800		330-97800		(29) 2002
Netherlands, river	10-190	70-2300	70-3100	10-1800	900-5000	<LOQ-80	(49) 2005
UK, river					360-21000		(50) 1998
USA, river			140-4410	40-350			(51) 2007
France, river		70-180	70-320		160-310		(37) 2009
France, river		330-480	50-140		120-640		(33) 2013
Netherlands, estuary			40-1880		50-4960		(5) 2006
Mersey Estuary UK					130-690		(52) 1989
Mississippi Estuary					70		(53) 1978
Nueces Estuary, Texas					210-770		(9) 1983

5.5 Conclusions

Phthalate esters were detected in samples of final effluent obtained from eleven CT LIS shoreline WWTPs, in the spring of 2012. DEHP was the predominant PAE detected, with concentrations measured ranging from 50 ng/L to 587 ng/L. DEP was detected in 100% of final effluent samples, in concentrations ranging from 3.2 ng/L to

41.8 ng/L. DMP and DnOP were detected in less than half of the final effluent samples, in concentrations ranging from <LOQ to 4 ng/L and <LOQ to 8.6 ng/L respectively. DEP, DEHP and DnOP were found associated with the SPM phase in concentration ratios reflective of those detected in the aqueous phase. DEP, DEHP and DnOP were detected associated with SPM fraction in the final effluents. DEHP was the predominant PAE associated with SPM, detected in 100% of samples and above LOQ in 73%, with concentrations ranging from <LOQ to 237.5 ng/L of effluent filtered.

Effluent concentrations of PAEs were measured again in the summer, focusing on the six WWTPs located in the lower Housatonic River Estuary. Five of the WWTPs in this location had been sampled earlier in the spring; Ansonia, Derby, Shelton, Stratford and Milford Housatonic. Effluent samples were again filtered to obtain both aqueous and efSPM fractions however the filtration blanks for the summer survey were compromised, therefore only aqueous or dissolved phase (operationally defined as passing through a nominal <0.7 μm GF/F filter) concentration measurements were obtained. Average dissolved phase concentrations of DEHP were however 6.7 times higher in the summer, with average dissolved phase DEHP concentrations increasing from 27.6 ng/L (16 – 51 ng/L min – max values) in the spring to 185.8 ng/L (39 – 452 ng/L min – max) in the summer, when considering only the four WWTPs Derby, Shelton, Milford Housatonic and Stratford. Dissolved DEHP concentrations measured in Ansonia were far greater than the other WWTPs in both spring and summer surveys; 349.8 ng/L in the spring, and 5575 ng/L in the summer, greater than the highest calibration standard of 1000 ng/L.

Partitioning coefficients derived for DEHP distribution between dissolved and efSPM phases in the final effluent streams (Koc) were greater than values previously reported. However, this factor is attributed to the low particulate concentrations; an important parameter that must be considered in the modeling of pollutant fate and transport since utilizing partitioning coefficients without consideration for the particulate concentration effect can lead to a substantial underestimation of SPM phase DEHP. Effluent derived SPM therefore plays an important role in the transfer of PAEs out of the wastewater treatment process and into receiving waters, and this is of particular concern since efSPM is a highly valuable food source to benthic biota, and has been shown to be preferentially assimilated (40). Additional laboratory studies are recommended to further investigate the partitioning of PAEs to efSPM, to determine a relationship between partitioning coefficients derived and efSPM concentration, to relate to the field based values derived in this study as well as for comparison to the similar study conducted by Turner and Rawling using riverine and estuarine SPM (22).

The fate of PAEs in the receiving waters of the Housatonic River estuary was investigated in the summer of 2012. DEHP and DEP were detected in the surface waters of the Housatonic River, and in the surface waters of the coastal LIS in the region of the Housatonic River plume. DEHP is hydrophobic in nature, with reported octanol-water partitioning coefficients ($\log K_{ow}$) ranging from 4.20 – 8.90 (4) and a suggested $\log K_{ow}$ value of 7.73 (54). In receiving waters DEHP is expected to sorb to solid phases; sediments are expected to be the main repository for DEHP. WWTP discharge was expected to result in loss to sediments in the vicinity of the input region, creating a local concentration gradient of DEHP in the sediment decreasing with

distance away from the input source. Surface water concentrations of DEHP along the Housatonic River salinity gradient did not appear to show conservative mixing along the river, as would be predicted with loss of DEHP to sediments. DEHP surface water concentrations were greater at river station 4 (80.8 ng/L) than at the upper river stations 8 and 9 near Derby, and the river station 2 located near Stratford, where DEHP concentrations ranged between 22 ng/L – 30 ng/L. This could signify a local source of DEHP to the river in the vicinity of River station 4, however additional along river surveys are recommended to verify the potential for DEHP inputs to this area of the river. River station 4 is located approximately 2000 meters downstream of the Milford Housatonic WWTP discharge zone; the concentrations of DEHP in the aqueous phase of the Milford Housatonic effluent were second only to Ansonia, measuring at 450 ng/L in the summer survey. While the particle reactive nature of DEHP would predict the loss of DEHP to sediments, particularly in the brackish waters of the Milford effluent discharge zone, the presence of a third colloidal phase was shown in both effluent waters, and within riverine and estuarine waters, to enhance the apparent solubility of DEHP, and therefore by extension, the transport of DEHP along the river in the dissolved phase may additionally be facilitated by partitioning to DOC.

Concentrations of DEHP were generally higher in surface waters in the shoreline of the LIS, indicating that the estuary could be a trap for this bioaccumulative PAE. The average DEHP concentrations measured in the estuary waters of 160 ng/L (26 – 228 ng/L range) are approaching the Environmental Risk Limit (ERL) for DEHP in water of 190 ng/L derived by van Wezel *et al.* (6) using chronic ecotoxicity data with relevant endpoints of survival, growth and reproduction, in aquatic organisms covering four

taxonomic groups. Additionally, with BCF values for fish species reported to range between 114 – 1380, average water phase DEHP could result in DEHP levels in estuary fish species of between 18 – 220 mg/kg. Future investigations are warranted to determine the risk of DEHP to aquatic species in this region.

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Chapter 6

Summary of Significant Findings and Recommendations for Future Research

6.1 Perfluoroalkyl Acids- Summary and conclusions

The occurrence and environmental fate of perfluoroalkyl acid (PFAA) surfactants in several Connecticut wastewater treatment plants and receiving waters was explored in the first part of this dissertation. Discharges of domestic and industrial origin have been identified as major sources of PFAAs to the aquatic environment in rivers (1, 2, 3) and coastal estuaries (4, 5). The initial goal of this research was to determine the presence of target PFAAs (C_4 - C_{12} carboxylic acids (PFCAs) and C_4 - C_{10} sulfonic acids (PFSAAs)) in CT wastewater effluent which discharges directly into the Long Island Sound (LIS) watershed, thereby establishing the first measurements, and therefore baseline concentrations, of PFAAs in the CT shoreline and LIS region.

Data on the occurrence of PFAAs in the final effluent of several CT WWTPs was presented in Chapter 3. PFOA was the predominant PFCA (detected in 100% of samples) contaminant in aqueous samples obtained spring 2012, with average (range) concentrations of 33.3 (6.3 - 64) ng/L, however PFPA and PFHxA were also detected at similar concentrations (25.9 (6.1 – 72.5) ng/L and 25.2 (6.2 - 47) ng/L respectively). The

trend towards shorter PFAA congeners was proposed to be a reflection of recent stewardship programs restricting the uses and applications of the longer chain ($C>7$) perfluoroalkyl compounds. The predominant PFSA detected was PFOS (observed in 80% of samples) at an average concentration of 18.2 ($<LOQ - 74.7$) ng/L. The annual total PFAA mass flow into the LIS, from an estimated 3.3×10^9 L per day of treated effluent, was estimated at 50 - 530 kg/year.

The occurrence and environmental fate of PFAAs entering receiving waters from WWTP effluent discharge was further investigated during several field surveys of the Housatonic River; the second largest river bringing freshwater to the LIS. Data presented in Chapter 4 revealed WWTP effluent to be a major source of PFAAs to the Housatonic River, as well as to the major tributary river to the Housatonic, the Naugatuck River. Investigations into the concentration and composition profiles of PFAAs in both wastewater effluent and in receiving waters strongly suggested that wastewater, mostly likely of domestic origin, was the major source of PFAAs to this watershed. Total PFAA concentrations in the Housatonic River and the river plume were also investigated during different hydrological regimes. Total PFAA concentrations decreased with increased river flow, suggesting that point sources such as WWTP effluent discharge were the predominant source of PFAAs to this river estuary system. Although measured concentrations were generally lower in river water samples during high volume river discharge, the volume flux of the river was 10 times higher; calculated overall total PFAA mass flow, based on the measured concentrations and the recorded river flow rate, were shown to have increased three times under high river flow conditions, suggesting that additional contributions of PFAAs from non-point sources,

such as precipitation and urban run-off, could not be excluded. Total PFAA mass flux from the Housatonic River to the Long Island Sound was estimated at 60 - 90 g/day in summer 2012 low flow, and 200 - 340 g/day under spring 2013 high river discharge hydrology.

The data presented in Chapter 4 for the summer low-river discharge survey (2012), and the follow up low-river discharge survey in October 2014 indicated that a stable concentration and composition profile signature detected in the Housatonic and Naugatuck Rivers strongly suggest that domestic origin WWTP derived effluent is the major PFAA source to this region. However, a large contamination event was measured in the effluent of one of the WWTPs located at the mouth of the Housatonic River during the spring high-river discharge survey in 2013. Concentrations of several PFSA, including the more bioaccumulative species PFOS and PFDS, were ~10-100 times greater than had previously been detected at that same location in both the spring and summer of the previous year. This data indicates that while major reductions in PFAA manufacture and use have resulted from the partnered industry-environmental agency stewardship measures, emission of restricted PFSA (such as PFOS) may still be of concern in this region, particularly as concentrations measured were approaching a reported Predicted No Effect Concentration (PNEC) for protecting marine wildlife (2.5 µg/L). Follow up measurements of PFSA levels in the Stratford WWTP effluent are warranted.

The distributions of PFAAs in the surface waters of the Housatonic River and in the plume of the river (as waters mixed with the estuarine waters of the LIS) were investigated during the summer low-river discharge survey. A trend of decreasing PFAA

concentrations towards the river mouth was seen, consistent with mixing of higher PFAA concentration up-river waters with low PFAA concentration salty estuary waters. Each PFAA was found to exhibit conservative mixing behavior along the salinity mixing line. The major portion of the PFAA mass discharged into the river system was therefore concluded to be conserved along the salinity gradient, and capable of being transported long distances, similar to what Huset *et al.* reported in the fresh water system of the Glatt River watershed (2). However, the presence of longer chained PFAAs ($>C_6$) was measured in bed sediments, particularly in close proximity to emission sources, in this case, WWTP discharge, as well as in the sediments of the river mouth estuary, specifically in the highly organic carbon rich region of the bordering marsh. Salt marsh areas of the Housatonic estuary were hypothesized to be catchment areas for longer chain PFAAs. The presence of PFAAs in these organic carbon rich areas may be cause for concern, due to the potential for bioavailability to benthic organisms. Sediments have been reported to be a major source of PFAAs to an aquatic food web (8), and sediment associated PFAAs have also been reported to be bioavailable (9); sorption to sedimentary materials does not necessarily constitute a sink for these contaminants.

Previously reported organic carbon partition coefficients ($\log K_{oc}$) as measured in field-based situations, for aqueous- suspended particulate matter (SPM) distributions, were >1 order of magnitude greater than the K_{oc} values reported for settling or bed sediments, in both laboratory and in field studies (5) (12) which indicate that particulate size difference may influence the sorption capacity. Given that SPM provides a crucial link for the movements of contaminants between water column, bed sediment and the

food chain, the elucidation of the partitioning dynamics between the dissolved and SPM phases was of specific interest in this work.

The potential for artifact arising from filtration was investigated and found to become significant ($> 10\%$ loss) for PFAAs with >8 perfluorinated carbons. The filter artifact was determined and applied, however, the partitioning coefficient ($\log K_{oc}$) values derived for partitioning between the effluent SPM and the aqueous phase in composite samples of final effluent, were 1-2 orders of magnitude greater than previously derived for sewage sludge in batch experiments (10). Partitioning was determined to be a function of chain length with a log unit increase in $\log K_{oc}$ values of ~ 0.4 log units per fluoroalkyl moiety, consistent with previous literature reports (5, 11, 12). PFOA was also measured in the SPM phase of river water samples obtained from 10 m downstream of the effluent discharge sources at Derby and Milford WWTPs. The SPM-water K_{oc} values derived for these river samples were consistent with those derived for efSPM-water partitioning of PFOA, therefore the samples obtained in these locations in close proximity of the WWTP effluent discharge were concluded to be samples of effluent origin and not riverine SPM. This observation also supported the high partitioning coefficients previously derived for PFOA with efSPM.

For riverine and estuarine samples, PFOS was the only PFAA detected in the SPM fraction along the salinity gradient of the Housatonic River and estuary in the summer low-river flow survey. $\log K_{oc}$ values derived in this case were also >2 orders of magnitude greater than those previously published, and did not vary with salinity, in contrast to the literature (3). Xiao *et al.* (13) reported a similar situation, and hypothesized the high partitioning values may be due to the presence of PFOS

containing particles from sources that could include debris of textiles, carpet and several industrial polymers. It may be possible, given the lack of variability of partitioning coefficients with salinity, that the SPM-PFOS measured in the Housatonic River is derived from PFOS containing particulates as described by Xiao *et al.* The lack of salinity relationship may be due to undersampling; however additional laboratory experiments conducted, using end-member waters mixed in different ratios, also failed to determine a salinity effect on partitioning coefficients at environmentally relevant PFOS concentrations. The higher PFOS concentrations were associated with SPM-organic matter with an isotopic ($\delta^{13}\text{C}$) signal consistent with an upper river terrestrial source. Additionally, the concentrations of PFOS in both riverine SPM and aqueous phases were higher in the upper river, and a linear sorption isotherm obtained for the data suggested active partitioning as opposed to particulate containing PFOS, which would not be actively partitioning. These observations support the hypothesis of active partitioning, and the higher partitioning coefficients derived in the field and laboratory for PFOS to SPM, indicating an important role for SPM in the fate and transport of PFOS in the river estuary system.

Longer chain PFAAs, which were shown a greater tendency to partition to the solid phase, and which (particularly PFDoA, PFTrA and PFTeA) were not detected in the water column, were detected in the Housatonic estuary by the use of oysters deployed as biomonitoring samplers. This result illustrates also an important role of SPM in the fate and transport of these less soluble PFAAs to the food chain.

6.2 Phthalic Acid Esters- Summary and conclusions

The global transport and fate of several phthalic acid esters (PAEs) has received widespread attention in recent years due to compounds in this class identified as endocrine disrupting chemicals. Of the 4.5 million tons of PAEs manufactured each year, approximately 50% is di-(2-ethylhexyl) phthalate (DEHP), and streambed sediment samples obtained from coastal basins in New England (1998-1999) show DEHP to be most prevalent PAE contaminant (14). The occurrence and partitioning behaviors of six phthalates; diethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-n-octyl phthalate (DnOP) and DEHP was investigated.

DEHP was found to be a contaminant in laboratory ultra-pure (MilliQ) water and the major PAE contaminant in wastewater final effluent from several CT WWTPs, followed by DEP. DEHP concentrations in final effluent ranged from <LOQ to >1000 (5575) ng/L. DEP concentrations ranged from <LOQ to 41.8 ng/L. A daily mass input of DEHP to the LIS, based on 3.3 trillion liters of treated effluent entering the LIS watershed daily, was estimated to be in the range of 175– 750 g/day; 60 - 270 kg/year. In final effluent samples, DEHP was found to associate with the dissolved and suspended particulate fractions. The K_{oc} value derived for effluent SPM-aqueous DEHP partitioning ($\log K_{oc} = 6.76 \pm 0.43$) was greater literature values ($\log K_{oc} = 4.94 - 5.71$) (15). However, using the regression equations derived by Turner and Rawling (16), the distribution coefficient for DEHP adsorption to particulates (in fresh water) as a function of the SPM concentrations measured in the WWTP final effluent, should be closer to a log value of 7. The apparent enhanced solubility of DEHP in final effluent due to the

presence of DOC illustrated that both SPM and DOC play a significant role in the transport of DEHP in the wastewater effluent stream, facilitating the transport of DEHP out of the WWTP and into receiving waters.

Finally, PAEs were detected in the receiving waters of the Housatonic River, and in the surface waters of the coastal LIS in the region of the Housatonic River plume. The average DEHP concentrations measured in the estuary waters of 160 ng/L (26 – 228 ng/L range) are approaching the Environmental Risk Limit (ERL) for DEHP in water of 190 ng/L derived by van Wezel *et al.* (17). These observations, in conjunction with previously published research regarding the behavior of DEHP in estuaries (16) suggest that the LIS estuary is a trap for this bioaccumulative PAE.

6.3 Recommendations for future research

Concentrations of PFAAs measured in final effluent samples from several CT WWTPs were found to be greater in WWTPs with higher discharge volumes and served a greater population. This raises important questions for further research regarding the potential PFAA input from other CT WWTPs not included in this survey, specifically WWTPs that serve much greater population densities with much higher daily flow rates, including the largest WWTP in CT located in Hartford. With the potential for PFAA loading to be greater in more heavily populated regions, the occurrence and potential impact of PFAAs in the western LIS, where reduced tidal flushing already results in numerous water quality issues including seasonal hypoxia, may also be an important area of future research for this regions ecosystems. Additional sources to the Sound,

including the impact of non-point sources such as run-off, and precipitation, should also be considered for further exploration.

As industrial emissions have been suggested to be the cause of an episodic PFOS polluting event in the Housatonic River estuary, future investigations should also be concerned with ascertaining the specific industrial practices which may have led to this high level PFSA discharge, whether it is common practice, and whether there are other potential sources from additional industrial sites that discharge into this ecosystem. The specific discharge zone where the high PFOS levels would have been released is directly adjacent to a salt marsh, as well as directly opposite another salt marsh that is a designated wildlife refuge. Because of the increased tendency of long chain PFAAs to partition preferentially to organic matter, possibly enhanced by the higher salinity and turbidity of this region, the potential of PFAAs to accumulate in these marsh areas is high, therefore future research should be concerned with the presence of these bioaccumulative and biomagnifying PFAAs in biota and sensitive wildlife species in the region, as well as an assessment made towards potential levels in seafood and the possible risk for human exposure.

The use of oysters as a biomonitoring species in this study was promising, enabling the identification of long-chain PFCAs not previously detected in either dissolved or SPM phases, particularly PFDoA and PFTrA, which were detected in oysters in the highest average concentrations. The results from this pilot study indicate that future research should include the use of oysters and other bivalves for pollution monitoring in this region. Another focus of attention should be on elucidating the sources of and input amounts to the aquatic environment of longer (>C₈) perfluoro-alkyl

chain PFAAs, and their presence within the food chain, as due to their high bioaccumulation and biomagnification potential, these compounds may be exerting negative effects on locally sensitive marine wildlife and ecosystems.

Results from the oyster study also imply an important role of SPM in the fate and transport of these less soluble PFAAs since these will be more likely associated with the SPM phase. The apparent enhanced partitioning to efSPM also warrants further examination, and additional laboratory partitioning experiments utilizing efSPM are recommended in order to verify the results obtained in the field. SPM phase PFOS was alternatively hypothesized to be due the presence of PFOS containing particles from the breakdown of consumer products, which would not be actively partitioning. Elucidating the mechanism behind the SPM-derived partitioning parameters from the field observations would be highly recommended for future investigations, as it may be that PFOS containing particles are not bioaccumulative, whereas PFOS enriched POM may be an effective vector of PFOS into the food chain. Understanding the nature of SPM phase PFOS would therefore be very important in assessing risk to biota and the potential for human exposure.

The role of SPM and in particular efSPM is a subject that is highly recommend by this study for future research attention, as efSPM has been reported to be preferentially assimilated by benthic biota (18) thus could result in high levels of contamination, and which may represent an important vector in the transport of the bioaccumulative/biomagnifying compounds of concern identified in this study, DEHP, PFOS and long-chain (>C₈) PFCAs, to the local food-web, including the potential levels

that may be in local LIS seafood and thus culminating in a possible concern for human exposure.

The results in this study on the occurrence and distributions of PAEs in WWTP effluent and receiving waters indicate that future research should also be focused on the role of effluent dissolved and particulate organic matter in the transportation of hydrophobic organic compounds through the wastewater treatment process and into receiving waters. In this study, the investigation into the role of SPM was hampered by filter contamination issues. Additional laboratory based experiments are warranted to determine the filtration artifact (the potential loss to filters of PAEs) and the SPM-water partitioning coefficient for PAEs in order to fully assess both the dissolved and particulate phase PAE loadings to the LIS. Finally, further research should address the relatively high concentrations of DEHP measured in the surface waters samples from the LIS region in the proximity of the Housatonic River mouth, to determine the potential of risk to aquatic ecosystems in this region. DEHP was detected in concentrations greater than those reported to elicit endocrine disruptive end-point in aquatic organisms. Further research to determine the impact of DEHP in the LIS is consequently recommended.

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APPENDIX

Table A1: UPLC parameters and running conditions

Instrument parameters:			
Capillary (kV)	3.00		
Source temperature	145°C		
Desolvation temperature	350°C		
Cone gas flow	50 L/Hr		
Desolvation gas flow	500 L/Hr		
Collision gas flow	0.10 mL/min		
Column temp	50°C		
UPLC gradient program:			
Time(min):	%A:	%B:	Curve:
0.00	85.0	15.0	Initial
1.50	85.0	15.0	6
7.00	15.0	85.0	6
7.10	0.0	100.0	6
8.00	0.0	100.0	6
9.00	85.0	15.0	6

A: 2 mM ammonium acetate in water/MeOH [95:5]

B: 2 mM ammonium acetate in MeOH

Table A2: MS ion transitions monitored for target compounds.

Analyte	Precursor ion [m/z]	Product ion [m/z]	Cone Volt [V]	Collision energy [V]	Recovery Standard used
PFBA	213	169	15.0	10.0	[¹³ C ₂]-PFHxA
PFPA	263	219	15.0	9.0	[¹³ C ₂]-PFHxA
PFHxA	313	269	13.0	10.0	[¹³ C ₂]-PFHxA
PFHpA	363	319	14.0	10.0	[¹³ C ₂]-PFHxA
PFOA	413	369	15.0	11.0	([¹³ C ₂]-PFHxA+[¹³ C ₂]-PFDA)/2
PFNA	463	419	15.0	11.0	[¹³ C ₂]-PFDA
PFDA	513	469	16.0	12.0	[¹³ C ₂]-PFDA
PFUnDA	563	519	18.0	12.0	[¹³ C ₂]-PFDA
PFDODA	613	569	18.0	13.0	[¹³ C ₂]-PFDA
PFTriDA	663	619	18.0	13.0	[¹³ C ₂]-PFDA
PFTeDA	713	669	19.0	14.0	[¹³ C ₂]-PFDA
PFBS	299	80	13.0	10.0	[¹⁸ O ₂]-PFHxS
PFHxS	399	80	50.0	38.0	[¹⁸ O ₂]-PFHxS
PFHpS	449	80	60.0	29.0	[¹⁸ O ₂]-PFHxS
PFOS	499	80	60.0	48.0	[¹⁸ O ₂]-PFHxS
PFDS	599	80	18.0	13.0	[¹⁸ O ₂]-PFHxS
Recovery Standards:					
[¹³ C ₂]-PFHxA	315	270	13.0	9.0	
[¹³ C ₂]-PFDA	515	470	16.0	12.0	
[¹⁸ O ₂]-PFHxS	403	84	50.0	38.0	
Instrument Standards:					
[¹³ C ₄]-PFOA	417.00	372.0	16.0	11.0	
[¹³ C ₄]-PFOS	502.80	79.9	60.0	48.0	

Table A3: Calibration parameters; limits of detection and quantitation determined by extrapolation from lowest calibration standard detected to the given S/N ratio (in ng/mL).
IDL=Instrument Detection Limit. LOD=Limit of detection.

PFAA	#Fluorinated carbons	Retention Time	Calibration Regression R^2	IDL (S/N=3)	LOD (S/N=10)
PFBA	3		0.9971	0.49	1.64
PFPA	4		0.9964	0.56	1.87
PFHxA	5		0.9844	0.26	0.88
PFHpA	6		0.9846	0.64	2.12
PFOA	7		0.9764	0.32	1.07
PFNA	8		0.9994	0.77	2.56
PFDA	9		0.9994	1.57	5.23
PFUnA	10		0.9893	0.45	1.51
PFDoA	11		0.9952	0.49	1.63
PFTriA	12		0.9997	4.61	15.35
PFTeA	13		0.9497	2.28	7.61
$^{13}\text{C}_2$-PFHxA	5		0.9985	0.11	0.36
$^{13}\text{C}_2$-PFDA	9		0.9845	2.04	6.82
PFBS	4		0.9907	0.08	0.29
PFHxS	6		0.9898	0.04	0.13
PFHpS	7		0.9933	0.15	0.50
PFOS	8		0.9951	0.17	0.56
PFDS	10		0.9946	0.14	0.46
$^{18}\text{O}_2$-PFHxS	6		0.9958	0.07	0.24

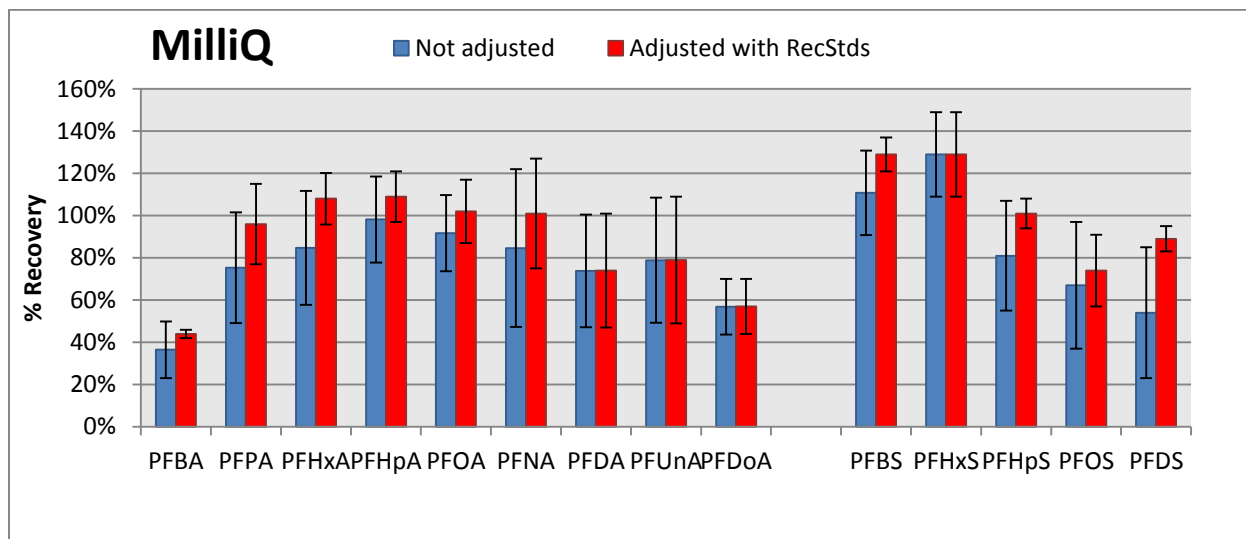


Figure A1: Recoveries of aqueous phase extraction: Samples of ultra-pure water (500 mL, n=4) were spiked with native target PFAAs (5ng) as well as isotope labeled recovery standards (5ng) prior to SPE extraction using HLB SPE columns. PFTrA and PFTeA were not detected above the LOQ (no peaks with S/N > 10).

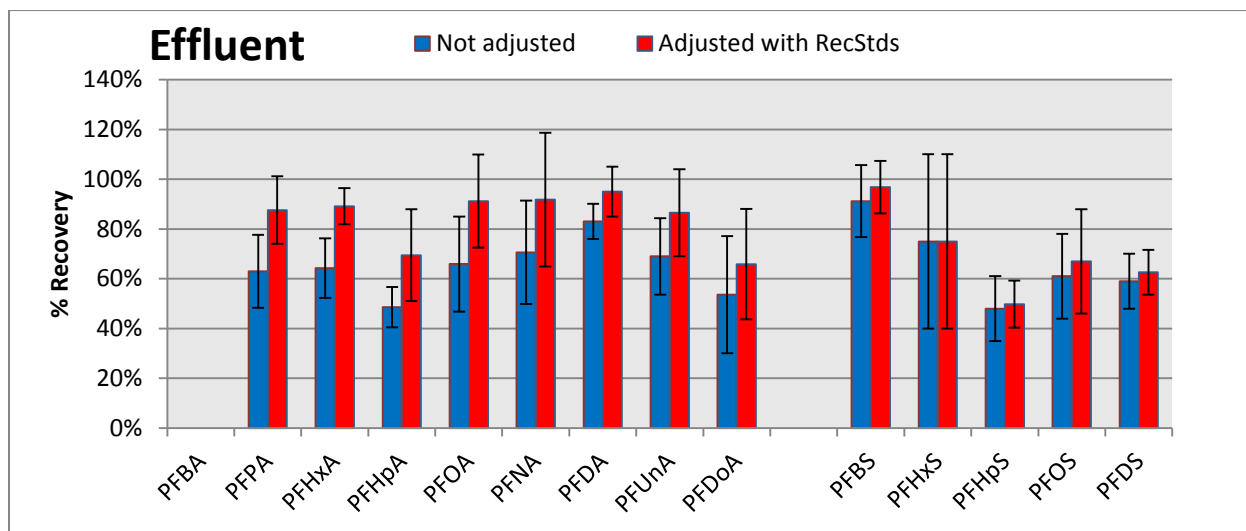


Figure A2: Recoveries of aqueous phase extraction: Samples of filtered effluent (500 mL, n=5) were spiked with native target PFAAs (5ng) as well as isotope labeled recovery standards (5ng) prior to SPE extraction using HLB SPE columns. PFBA, PFTrA and PFTeA were not detected above the LOQ (no peaks with S/N > 10).

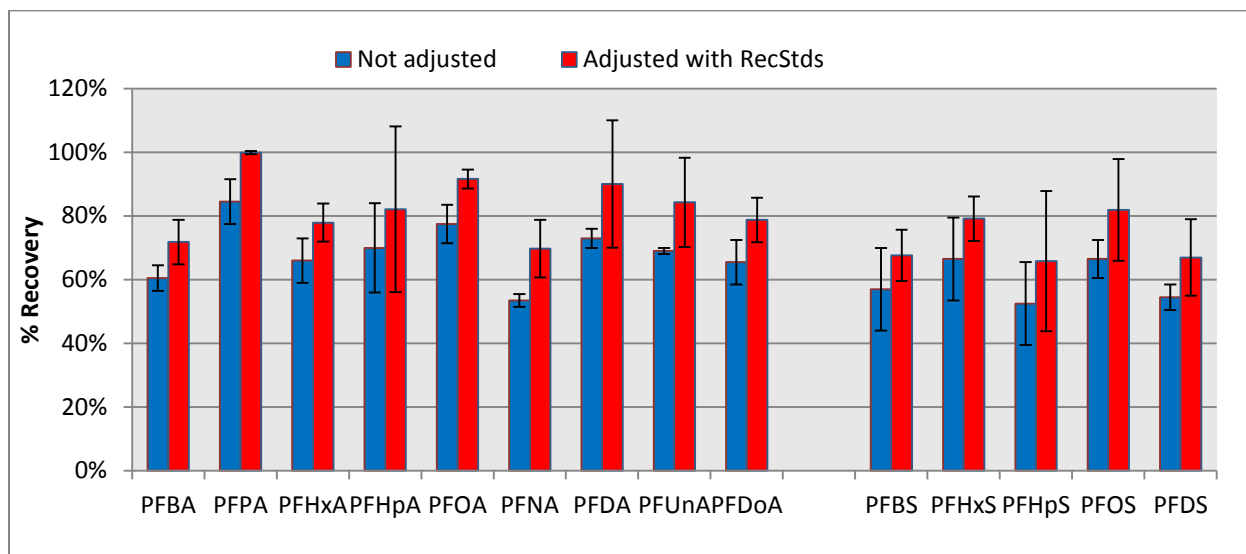


Figure A3: Recoveries of solid (SPM) phase extraction- spring survey: Blank PP filters (n=2) were spiked with native target PFAAs (5ng) as well as isotope labeled recovery standards (5ng) prior to extraction. PFBA, PFTrA and PFTeA were not detected above the LOQ (no peaks with S/N > 10).

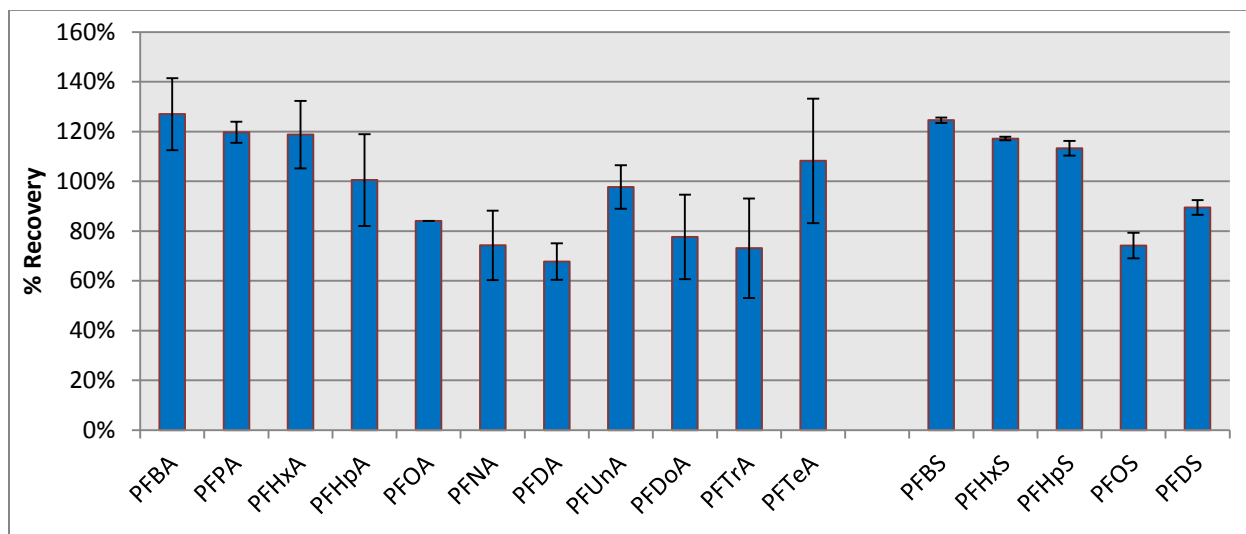


Figure A4: Recoveries of solid (SPM) phase extraction-summer survey: Blank PP filters (n=2) were spiked with native target PFAAs (10ng) as well as isotope labeled recovery standards (10ng) prior to extraction. All recoveries 95%-100% therefore no adjustments made.

Table A4: Average% recoveries (with standard deviation, or range of duplicates) of spiked samples processed with samples obtained during the spring and summer WWTP surveys: spiked blank PP filters for SPM extraction (n=2), and spiked ultra-pure water for SPE extraction (HLB (n=4) and WAX (n=1 (due to one sample loss)) columns) of aqueous phase. All samples adjusted for recovery using mass labeled recovery standards, except for the PP-SPM summer extractions, as all recovery standards showed 95%- 100% recoveries obtained. AE= analysis error, therefore not detected.

Extraction:	PFCAs:											PFSA:				
	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C4	C6	C7	C8	C10
PP-SPM (Spring)	72 (7)	100 (1)	78 (6)	82 (26)	92 (3)	70 (9)	90 (20)	84 (14)	79 (7)	AE	AE	68 (8)	79 (7)	66 (22)	82 (16)	67 (12)
PP-SPM (summer)	127 (15)	120 (4)	119 (14)	101 (19)	84 (0)	74 (14)	68 (7)	98 (9)	78 (17)	73 (20)	108 (25)	125 (1)	117 (1)	113 (3)	74 (5)	89 (3)
HLB SPE (Spring)	44 (2)	96 (19)	108 (12)	109 (12)	102 (15)	101 (26)	74 (27)	79 (30)	57 (13)	A.E.	A.E.	129 (8)	129 (20)	101 (7)	74 (17)	89 (6)
WAX SPE (summer)	116	107	110	105	110	94	93	112	107	51	65	129	116	125	85	87

Table A5: Detailed information concerning the amount of recovery standards detected in samples. Samples with recovery standards recovered in the range of 70%-130% were not adjusted. Samples with recovery standards <70% were adjusted up to 70% recovery. Samples with recovery standards >130% were adjusted down to 100% recovery.

Survey	n	Sample/extraction	% Recovery of mass labeled standards		
			%Standard Deviation		
			%max-%min		
			¹³ C-PFHxA	¹³ C-PFDA	¹⁸ O-PFHxS
WWTP- spring	24	Aqueous/SPE	68	99.3	84.5
			16.7	28.3	23.7
			42-105	57.5-166	37-119
WWTP- spring	14	Solid/SPM-PP filter	63	81.6	71.6
			13	15.9	12.9
			45-90	56-103	56.2-92.3
WWTP- summer	12	Aqueous/SPE	85.7	52.4	67.3
			28.7	25.9	15.3
			59-142	25-102	42-94
WWTP-summer	8*	Solid/SPM-PP filter	91.2	79.3	90.9
			10.8	19.5	17.0
			87-105	60-119	56-106

*4 samples had elevated amounts of recovery standards ranging from 166%-392% for ¹³C-PFHxA, 116%-450% for ¹³C-PFDA, and 153%-299% ¹⁸O-PFHxS. These higher recoveries were assumed to be a result of matrix effect therefore all PFAAs detected in these samples were adjusted down to 100% recovery.

Table A6: WWTP effluent survey data- PFAA concentrations (ng/L) measured in the dissolved (aq) and suspended particulate matter (SPM) phases of final effluent obtained from 11 WWTPs, spring 2012. Values adjusted for recoveries using isotopically labeled PFAA recovery standards. All dissolved phase samples were performed in duplicate (n=2) unless otherwise stated, with the range between the duplicate values; n=1 for SPM samples. <LOD = no peak with S/N >3; <LOQ = peak detected with S/N >3 and <6. Values given in parenthesis indicate peaks detected were above LOQ but below MDL (S/N <10). No filter artifact correction has been applied for this data.

WWTP	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFHpS	PFOS	PFDS
A (aq)	<LOD	37.6	38.3	20.9	36.1	10.4	<LOD	<LOD	<LOD	6.8	18.4	<LOD	19.8	<LOD
-range		0.7	3.4	10.5	4.0	0.8				0	2.4		0.2	
A (SPM)	<LOD	<LOD	<LOQ	<LOD	2.7	<LOD	2.0	0.9	0.5	<LOD	1.2	<LOD	6.7	<LOD
B (aq)	<LOQ	26.7	37.4	17.3	52.7	(11.6)	<LOD	<LOD	<LOD	4.2	3.2	<LOD	7.6	<LOD
-range		7.8	8.3	3.0	8.5	5.8				0.6	1.2		3.8	
B (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C- Composite (aq)	<LOD	<LOD	<LOD	7.0	15.0	<LOD	<LOD	<LOD	<LOD	3.7	NC	<LOD	7.6	<LOD
-range				0.4	8.7					0.7	NC		5.0	
C- Composite (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NC	<LOD	<LOD	<LOD
C- Grab (aq) (n=1)	<LOD	<LOD	<LOD	6.1	11.2	<LOD	<LOD	<LOD	<LOD	4.1	NC	<LOD	4.9	<LOD
C- Grab (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NC	<LOD	<LOD	<LOD
D (aq)	<LOD	21.1	28.6	16.1	29.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	19.0	<LOD
-range		0.5	1.0	4.0	4.6								0.5	
D (SPM)	<LOD	<LOD	<LOD	<LOD	3.8	<LOD	<LOD	<LOD	<LOD	<LOD	2.9	<LOD	3.1	<LOD
E (aq)	<LOD	23.5	21.5	16.1	33.2	13.5	<LOD	<LOD	<LOD	(4)	<LOD	<LOD	2.8	<LOD
-range		0.3	3.2	2.4	8.4	6.8				0			0.8	
E (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.1	7.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
F (aq)	<LOD	(8.8)	23.4	12	25.6	15.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4	<LOD
-range		1.2	1.4	6		6.4							0.8	
F (SPM)	<LOD	<LOD	<LOQ	<LOD	<LOD	2.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
G- Composite (aq)	<LOD	6.5	6.9	6.1	16.1	8.7	<LOD	<LOD	<LOD	5.0	NC	<LOD	<LOD	<LOD
-range		0.4	3.5	1.9	1.1	3.3				0.2	NC			
G- Composite (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NC	<LOD	<LOD	<LOD
G- Grab (aq) (n=1)	<LOD	7.2	6.2	11.3	10.5	<LOD	<LOD	<LOD	<LOD	6.9	NC	<LOD		<LOD
G- Grab (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NC	<LOD	<LOD	<LOD
H (aq)	<LOD	14.4	26.2	(10.8)	23.4	(12.8)	(1.9)	<LOD	<LOD	(3.6)	<LOD	<LOD	0.6	<LOD
-range		1.2	0.2	5.4	1.4	1.2	0.8			1.3			0.2	
H (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.4	<LOD	<LOD	<LOD
I (aq)	<LOD	27.2	38.4	17.6	38.9	13.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.6	<LOD
-range		11.2	8.6	0.6	0.3	7.8							1.8	
I (SPM)	<LOD	<LOD	<LOD	<LOD	2.5	<LOD	<LOD	1.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
J (aq)	<LOD	45.4	31.0	24.0	43.0	13.6	7.2	<LOD	<LOD	7.4	28.4	<LOD	16.0	<LOD
-range		1.4	1.4	5.1	17.3	2.9					14.2		8.5	
J (SPM)	<LOD	<LOD	<LOD	<LOD	4.8	<LOD	<LOD	<LOD	<LOD	<LOD	2.1	<LOD	7.3	<LOD
K (aq)	<LOD	30.8	33.7	19.4	59.8	10	6	<LOD	<LOD	3.6	18.8	<LOD	3.2	<LOD
-range		1.2	5.3	0.2	1.8	0	3			0.8	9.4		1.6	
K (SPM)	<LOD	<LOD	<LOQ	<LOQ	2	1.3	(1.0)	<LOD	<LOD	<LOD	0.7	<LOD	2.2	<LOD

Table A7: WWTP effluent survey data- PFAA concentrations (ng/L) measured in the dissolved (aq) and suspended particulate matter (SPM) phases of final effluent obtained from 6 WWTPs. Values adjusted for recoveries using isotopically labeled PFAA recovery standards. All dissolved phase samples were performed in duplicate (n=2) unless otherwise stated, with the range given for the duplicate values; n=1 for SPM samples. <LOD = no peak with S/N >3; <LOQ = peak detected with S/N >3 and <6. Values given in parenthesis indicate peaks detected were above LOQ but below MDL (S/N <10). No filter artifact correction has been applied to this data.

17TH

WWTP	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFHpS	PFOS	PFDS
A (aq)	13.1	59.3	22.6	7.4	34.7	8.8	(5.4)	(1.2)	<LOD	21.5	5.6	<LOD	29.6	(3.5)
-range	1.3	2.9	1.4	0.9	5.7	2.0	2.6	0.6		5.2	2.5		4.4	
A (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.4	<LOD
C (aq)	<LOD	17.6	21.6	7.9	17.3	4.5	<LOD	<LOD	<LOD	7.4	7.5	(2.3)	43.0	<LOD
-range		1.2	2.1	0.7	1.3	0.1				0.1	2.5	1.2	0.2	
C (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.7	<LOD	<LOD	<LOD	0.9	<LOD	11.3	<LOD
G (aq)	<LOD	22.8	16.9	8.5	24.5	23.5	(1.9)	(1.6)	<LOD	10.1	5.6	2.8	17.7	<LOD
-range		0.4	2.0	1.8	5.5	0.9	1.0	0.8		1.5	0.6	0.3	0.4	
G (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.9	<LOD	<LOD	<LOD	(0.8)	<LOD	1.9	<LOD
I (aq)	13.6	15.4	11.4	5.6	22.1	11.6	(7.3)	<LOD	<LOD	<LOD	3.8	<LOD	6.9	<LOD
-range	4.4	4.0	3.4	0.9	9.1	4.8	3.6				0.6		0.6	
H (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	(0.7)	2.4	<LOD	<LOD	<LOD	<LOD	<LOD	3.7	<LOD
K (aq)	10.5	30.8	19.1	7.3	17.7	10.4	(4)	2.1	<LOD	11.5	8.1	3.9	22.3	<LOD
-range	1.3	0.2	0.8	0.5	2.2	2.2	2	0.4		4.6	0.4	0.3	3.1	
K (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	(0.5)	(1.5)	<LOD	<LOD	<LOD	(0.8)0	<LOD	4.1	<LOD
L (aq)	(10.3)	16.6	15	8.4	14.6	14.6	(0.6)	1.4	<LOD	8.2	4.7	<LOD	14.6	<LOD
-range	5.2	0.7	0.7	0.8	2.4	2.0	0.3	0		0.4	0.6		0.3	
L (SPM)	<LOD	<LOD	<LOD	<LOD	<LOD	1.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.9	<LOD

23RD

WWTP	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFHpS	PFOS	PFDS
A (aq)	14.3	72.5	25.4	7.0	42.2	12.4	<LOD	<LOD	<LOD	24.7	4.2	2.8	14.2	<LOD
-range	0.7	2.6	0.8	0.6	2.5	0.3				8.3	0.6	0	1.2	
A (SPM)	<LOD	<LOD	<LOD	<LOD	3.0	(0.9)	0.5	<LOD	<LOD	<LOD	<LOD	<LOD	9.2	1.3
C (aq)	21.2	32.3	30.4	11.2	42.0	6.0	13	<LOD	<LOD	16.7	10.1	<LOD	40.5	<LOD
-range	1.2	0.1	0.2	1.4	0.8	0	0.9			2.0	0.8		2.3	
C (SPM)	<LOD	<LOD	<LOD	<LOD	1.5	<LOD	4.1	<LOD	<LOD	<LOD	<LOD	<LOD	34.2	0.8
G (aq)	11.0	34.0	22.9	6.9	44.9	42.8	(4.4)	<LOD	<LOD	9.3	7.8	<LOD	22.0	<LOD
-range	0.9	3.2	3.9	0.1	2.9	8.5	2.2			2.1	0.8		7.7	
G (SPM)	<LOD	<LOD	<LOD	<LOD	(1.1)	3.3	3.7	<LOD	(1.2)	<LOD	<LOD	<LOD	15.7	(0.4)
I (aq)	18.4	20.4	15.7	8.0	34.8	11.0	4.8	<LOD	<LOD	(4.6)	3.9	<LOD	8.3	<LOD
-range	1.4	0.8	0.1	1.0	6.5	0.8	0.2			2.3	0.3		0.3	
H (SPM)	<LOD	<LOD	<LOD	<LOD	(1.4)	1.5	3.5	<LOD	(1.2)	<LOD	<LOD	<LOD	<LOD	1.0
K (aq)	11.5	31.8	21.6	9.3	17.7	16.3	<LOD	2.9	<LOD	(9.4)	6.3	3.2	12.3	<LOD
-range	2.9	0.4	1.6	0.5	0	1.4		0.6		4.7	1.5	0	0.1	
K (SPM)	<LOD	<LOD	<LOD	<LOD	2.1	3.9	3.5	(5.7)	<LOD	<LOD	(1.4)	<LOD	8.1	<LOD
L (aq)	11.1	11.2	12.6	7.6	30.0	26.5	<LOD	3.3	<LOD	<LOD	7.2	<LOD	13.5	<LOD
-range	1.7	0.4	0.7	0.6	0.2	1.5		0.5			1.2		0.8	
L (SPM)	<LOD	<LOD	<LOD	<LOD	3.2	7.8	7.2	1.8	<LOD	<LOD	1.4	<LOD	14.7	(1.0)

Table A8: MS ion transitions monitored for target compounds (Housatonic River survey spring 2013 and all subsequent analysis).

Analyte	Precursor ion [m/z]	Product ion [m/z]	Cone Volt [V]	Collision energy [V]	Recovery Standard used
PFBA	213	169	15	10	[¹³ C ₄]-PFBA
PFPA	263	219	15	9	([¹³ C ₄]-PFBA+[¹³ C ₂]-PFHxA)/2
PFHxA	313	269	13	10	[¹³ C ₂]-PFHxA
PFHpA	363	319	14	10	([¹³ C ₂]-PFHxA+[¹³ C ₄]-PFOA)/2
PFOA	413	369	15	11	[¹³ C ₄]-PFOA
PFNA	463	419	15	11	[¹³ C ₅]-PFNA
PFDA	513	469	16	12	[¹³ C ₂]-PFDA
PFUnDA	563	519	18	12	[¹³ C ₂]-PFUnA
PFDODA	613	569	18	13	[¹³ C ₂]-PFDODA
PFTriDA	663	619	18	13	[¹³ C ₂]-PFDODA
PFTeDA	713	669	19	14	[¹³ C ₂]-PFDODA
PFBS	299	80	13	10	[¹⁸ O ₂]-PFHxS
PFHxS	399	80	50	38	[¹⁸ O ₂]-PFHxS
PFHpS	449	80	60	29	([¹⁸ O ₂]-PFHxS+[¹³ C ₄]-PFOS)/2
PFOS	499	80	60	48	[¹³ C ₄]-PFOS
PFDS	599	80	18	13	[¹³ C ₄]-PFOS
Recovery Standards:					
[¹³ C ₄]-PFBA	217	172	15	10	
[¹³ C ₂]-PFHxA	315	270	13	9	
[¹³ C ₄]-PFOA	417	372	15	11	
[¹³ C ₅]-PFNA	468	423	15	11	
[¹³ C ₂]-PFDA	515	470	16	12	
[¹³ C ₂]-PFUnA	565	520	18	12	
[¹³ C ₂]-PFDODA	615	570	18	13	
[¹⁸ O ₂]-PFHxS	403	103	50	38	
[¹³ C ₄]-PFOS	503	99	60	48	

Table A9: Calibration parameters: Limit of detection and quantitation determined by extrapolation from lowest calibration standard S/N ratio (in ng/mL) for analysis summer 2012 (Housatonic River (HR) survey 1- low river discharge, including the six HR WWTPs, oyster and sediment extractions. *Value determined by Quanlynx from calibration.

PFAA	#Fluorinated carbons	Retention time	Calibration Regression R ²	IDL (S/N=3)	LOQ (S/N=10)
PFBA	3	1.34	0.9949	2.6*	4.2
PFPA	4	3.70	0.9936	2.4*	3.9
PFHxA	5	4.99	0.9901	1.5*	1.8
PFHpA	6	5.71	0.9944	1.7*	2.0
PFOA	7	6.21	0.9991	0.1	0.3
PFNA	8	6.59	0.9871	1.5*	2.0
PFDA	9	6.93	0.9983	0.1	0.5
PFUnA	10	7.20	0.9894	0.5*	0.9
PFDoA	11	7.42	0.9906	1.0*	1.8
PFTriA	12	7.58	0.9938	0.5	1.8
PFTeA	13	7.72	0.9748	0.7*	1.5
¹³C₂-PFHxA	5	5.01	0.9970	0.4*	0.6
¹³C₂-PFDA	9	6.92	0.9985	0.4*	0.9
PFBS	4	4.21	0.9972	1.0*	1.4
PFHxS	6	5.78	0.9981	0.4*	0.6
PFHpS	7	6.23	0.9855	1.3*	1.5
PFOS	8	6.61	0.9949	1.9*	2.1
PFDS	10	7.21	0.9915	1.0*	1.2
¹⁸O₂-PFHxS	6	5.79	0.9975	0.06*	0.1

Table A10: Calibration parameters- serial dilution calibration standards curve. Limit of detection and quantitation determined by extrapolation from lowest calibration standard to the indicated S/N ratio (value given in ng/mL) for analysis spring 2013 (Housatonic River (HR) survey 2- high river discharge.

PFAA	#Fluorinated carbons	Retention time	Calibration Regression R^2	IDL (S/N=3)	LOQ (S/N=10)
PFBA	3	1.23	0.9996	0.4	1.2
PFPA	4	3.47	0.9998	0.7	2.2
PFHxA	5	4.63	0.9978	0.3	1.0
PFHpA	6	5.43	0.9966	0.3	1.2
PFOA	7	5.97	0.9941	0.3	1.0
PFNA	8	6.41	0.9939	0.4	1.4
PFDA	9	6.74	0.9972	0.2	0.7
PFUnA	10	7.03	0.9934	0.3	0.9
PFDoA	11	7.26	0.9970	0.9	2.8
PFTriA	12	7.47	0.9840	0.7	2.4
PFTeA	13	7.64	0.9962	1.9	6.3
[$^{13}\text{C}_4$]-PFBA	3	1.24	0.9987	0.1	0.4
[$^{13}\text{C}_2$]-PFHxA	5	4.64	0.9984	0.3	0.8
[$^{13}\text{C}_4$]-PFOA	7	5.97	0.9950	0.3	1.0
[$^{13}\text{C}_5$]-PFNA	8	6.39	0.9952	0.4	1.4
[$^{13}\text{C}_2$]-PFDA	9	6.74	0.9995	0.3	1.2
[$^{13}\text{C}_2$]-PFUnA	10	7.03	0.9885	0.2	0.8
[$^{13}\text{C}_2$]-PFDoA	11	7.28	0.9732	0.3	1.1
PFBS	4	3.95	0.9994	0.2	0.7
PFHxS	6	5.53	0.9966	0.4	1.3
PFHpS	7	6.01	0.9908	0.4	1.3
PFOS	8	6.41	0.9805	0.2	0.7
PFDS	10	7.01	0.9705	0.3	1.1
[$^{18}\text{O}_2$]-PFHxS	6	5.51	0.9985	0.3	1.2
[$^{13}\text{C}_4$]-PFOS	8	6.41	0.9852	0.2	0.7

Table A11: Calibration parameters: Limit of detection and quantitation determined by extrapolation from lowest calibration standard to the indicated S/N ratio (value given in ng/mL) for analysis of 2014 samples- Housatonic River (HR) survey 3 and laboratory experiments on partitioning.

PFAA	Retention time	Serial curve (SPM samples)			Extracted curve (SPE samples)		
		Calibration Regression R ²	IDL (S/N=3)	LOQ (S/N=10)	Calibration Regression R ²	IDL (S/N=3)	LOQ (S/N=10)
PFBA	1.23	0.9989	0.7	2.2	0.9972	0.2	0.6
PFPA	3.47	0.9996	0.6	2.1	0.9965	0.2	0.6
PFHxA	4.63	0.9997	0.3	0.7	0.9928	0.1	0.5
PFHpA	5.43	0.9969	0.4	1.3	0.9979	0.2	0.6
PFOA	5.97	0.9993	0.3	0.9	0.9874	0.4	1.3
PFNA	6.41	0.9988	0.2	0.7	0.9880	0.4	1.3
PFDA	6.74	0.9894	2.2	2.7	0.9904	0.7	2.2
PFUnA	7.03	0.9976	0.2	0.6	0.9937	0.4	3.3
PFDaA	7.26	0.9364*	1.2	1.8	0.9766	0.5	5.0
PFTriA	7.47	0.9968	0.4	1.4	0.9869	2.9	4.8
PFTeA	7.64	0.9923	0.1	0.5	0.9709	4.0	7.2
[¹³ C ₄]-PFBA	1.24	0.9994	0.2	1.6	0.9951	0.9	2.9
[¹³ C ₂]-PFHxA	4.64	0.9972	0.2	0.7	0.9982	0.1	0.3
[¹³ C ₄]-PFOA	5.97	0.9978	0.3	1.1	0.9980	0.3	1.0
[¹³ C ₅]-PFNA	6.39	0.9975	0.4	1.3	0.9922	0.2	0.7
[¹³ C ₂]-PFDA	6.74	0.9941	0.5	1.6	0.9971	0.9	2.9
[¹³ C ₂]-PFUnA	7.03	0.9934	0.2	0.6	0.9958	0.9	3.1
[¹³ C ₂]-PFDaA	7.28	0.9926	1.0	3.5	0.9801	1.7	5.7
PFBS	3.95	0.9995	0.2	0.5	0.9942	0.1	0.2
PFHxS	5.53	0.9966	0.2	0.7	0.9923	0.1	0.2
PFOS	6.41	0.9945	0.1	0.4	0.9836	0.3	1.0
PFDS	7.01	0.9910	0.1	0.3	0.9923	0.1	0.3
[¹⁸ O ₂]-PFHxS	5.51	0.9926	0.1	0.2	0.9915	0.1	0.2
[¹³ C ₄]-PFOS	6.41	0.9971	0.1	0.3	0.9823	0.1	0.3

* Calibration curve did not produce correct QC check standard values; mass-labeled analogue (mPFDaA) calibration curve was used for sample analysis.

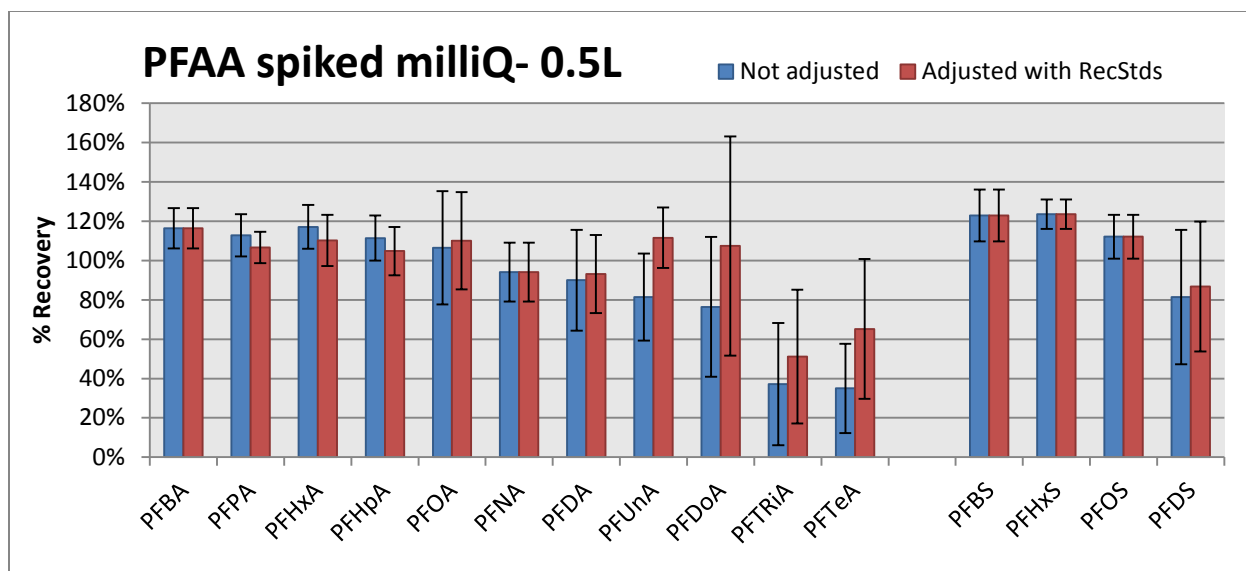


Figure A5: Recoveries of aqueous phase extraction: Samples of ultra-pure water (500 mL, n=7) were spiked with native target PFAAs (10 ng) as well as isotope labeled recovery standards (10 ng, Wellington labs, MPFAC-MXA) prior to SPE extraction using WAX SPE columns. Data was analyzed using calibration curve generated from SPE extracted calibration standards.

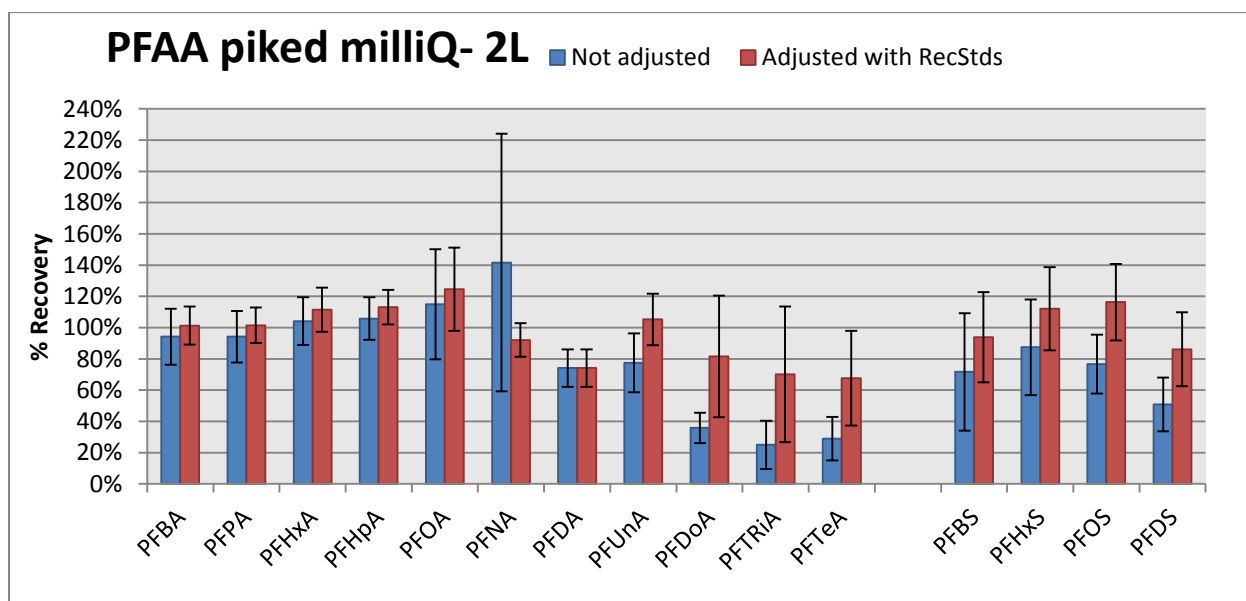


Figure A6: Recoveries of aqueous phase extraction: Samples of ultra-pure water (2 L, n=7) were spiked with native target PFAAs (10 ng) as well as isotope labeled recovery standards (10 ng, Wellington labs, MPFAC-MXA) prior to SPE extraction using WAX SPE columns. Data was analyzed using calibration curve generated from SPE extracted calibration standards.

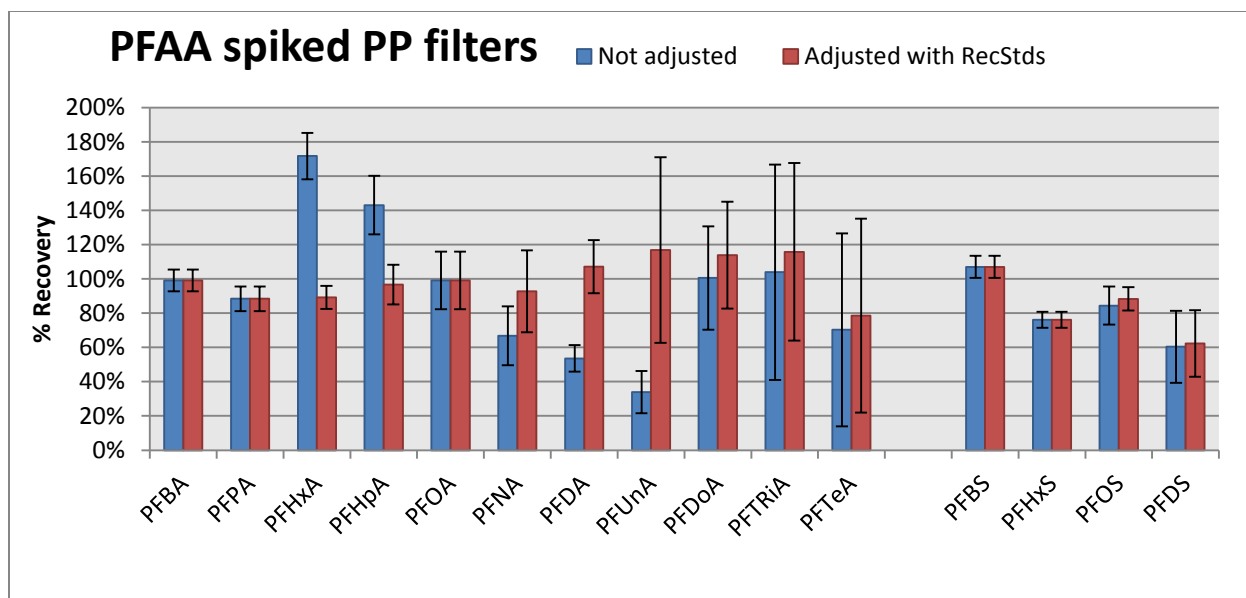
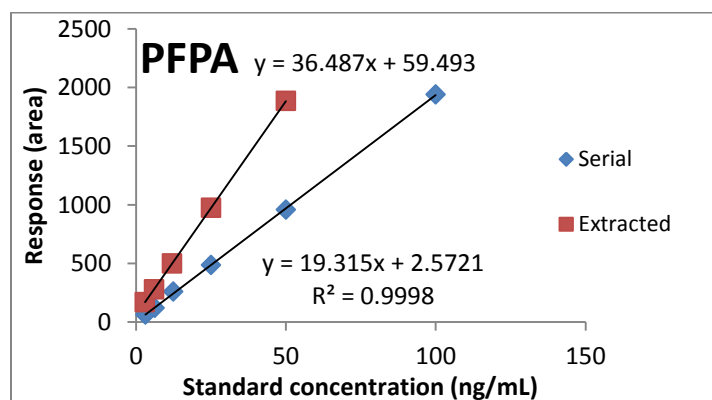
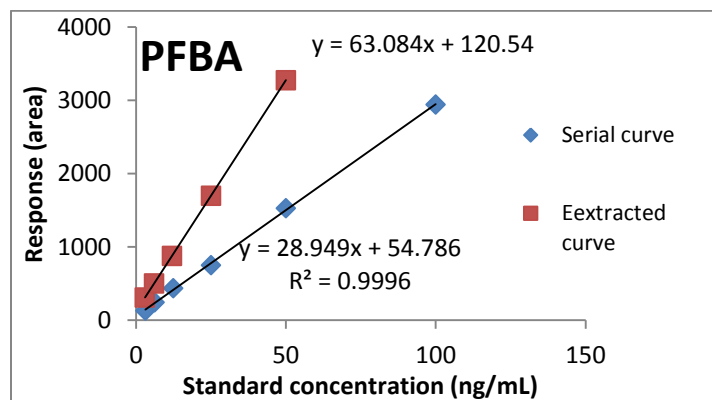
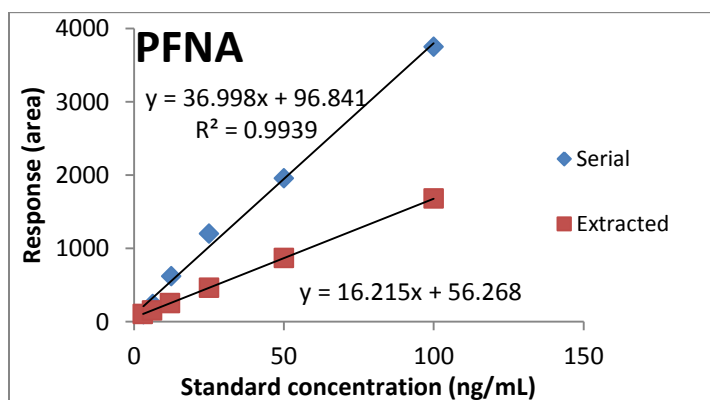
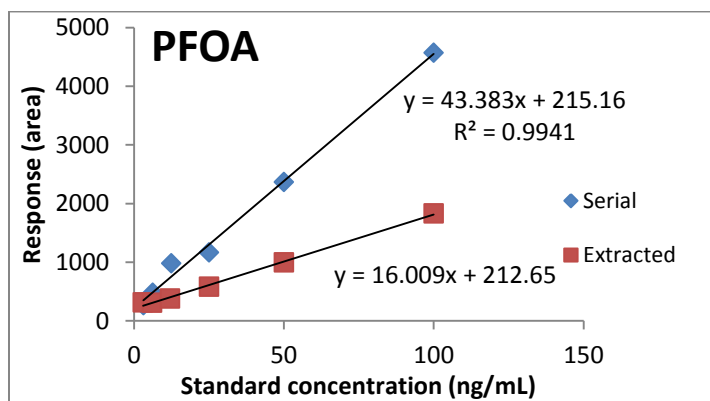
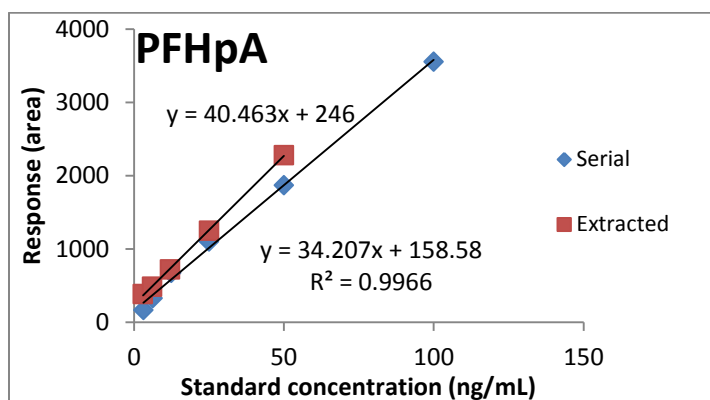
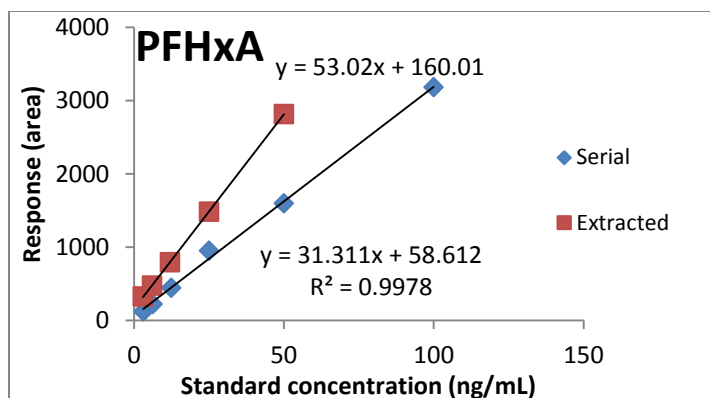
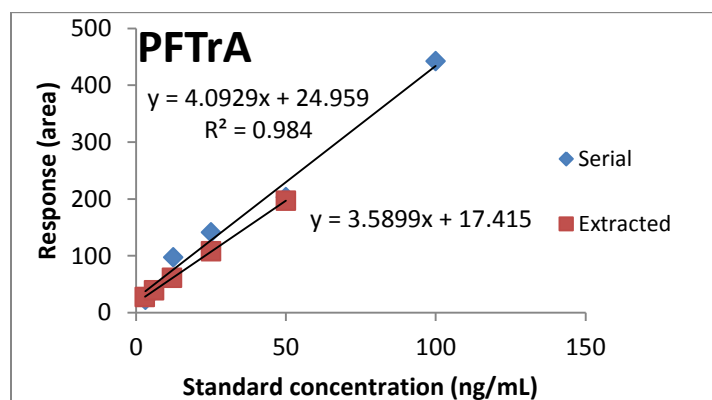
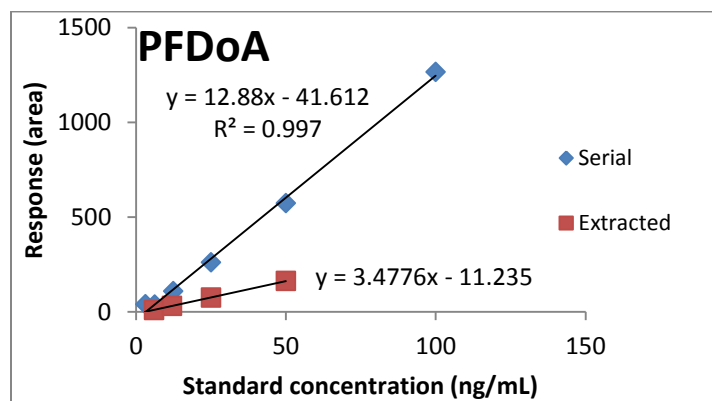
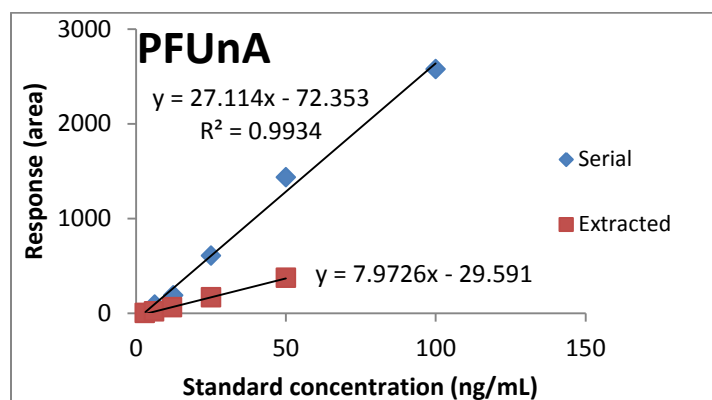
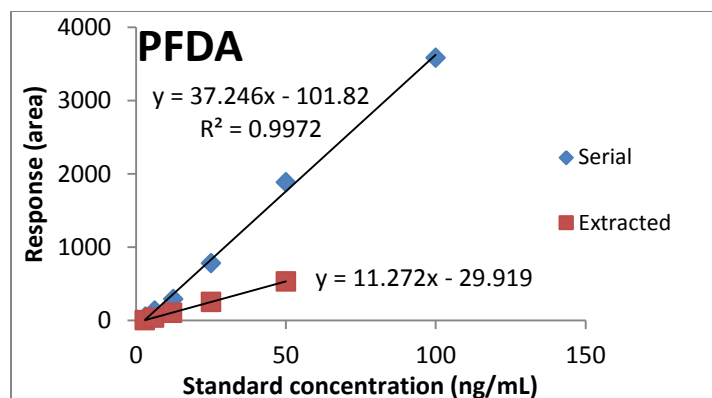


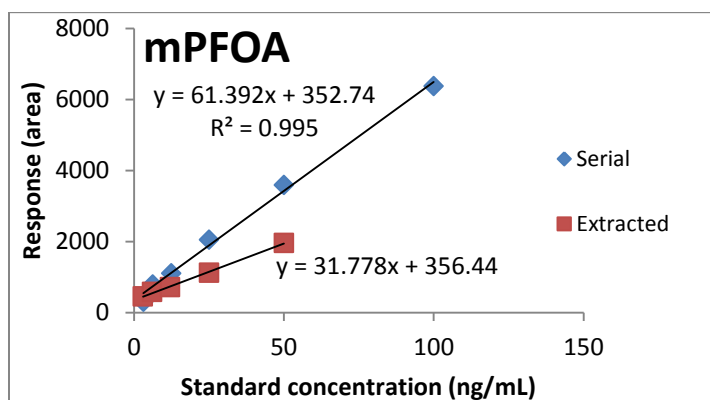
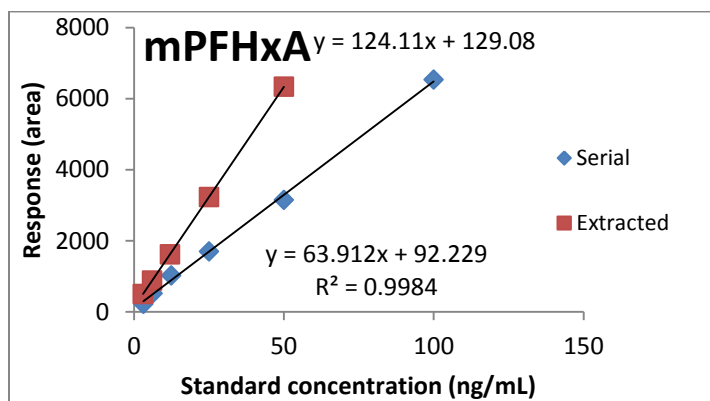
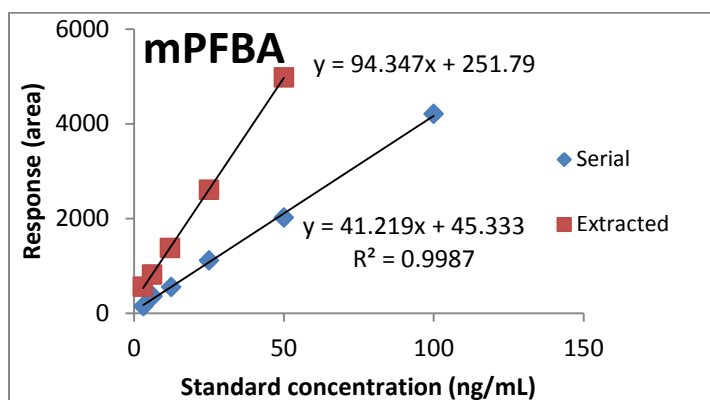
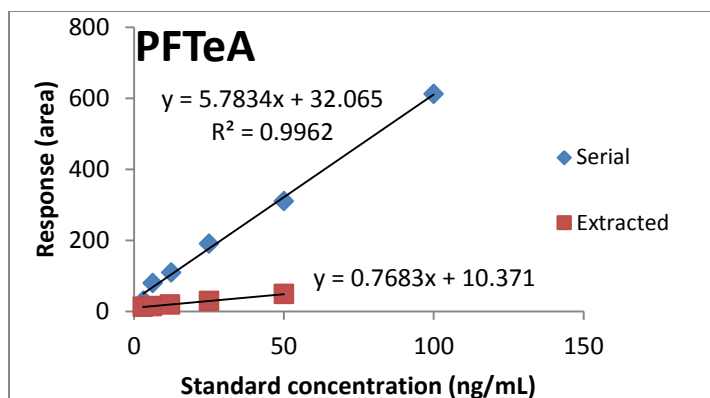
Figure A7: Recoveries of solid (SPM) phase extraction- summer Housatonic River survey: Blank PP filters (n=7) were spiked with native target PFAAs (10 ng) as well as isotope labeled recovery standards (10 ng) prior to extraction. Recoveries and standard deviations for PFCAs C₄ – C₁₀ and PFSA are within acceptable ranges. Longer chained PFCAs (>C₁₀) give better recoveries once adjusted with recovery standards, but are also much more prone to variability in the method. Data was analyzed using a calibration curve generated by serial dilution of calibration standards.

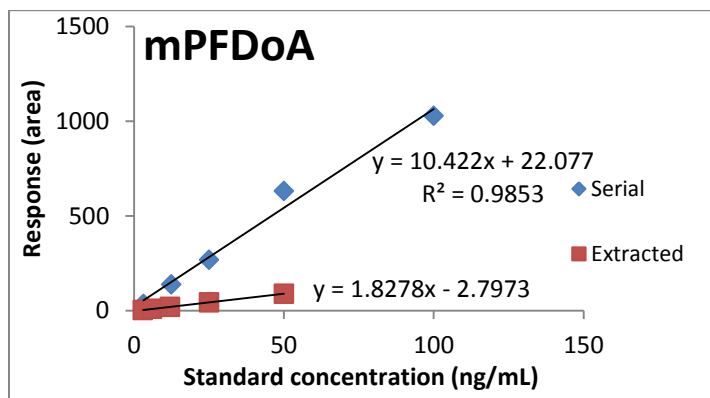
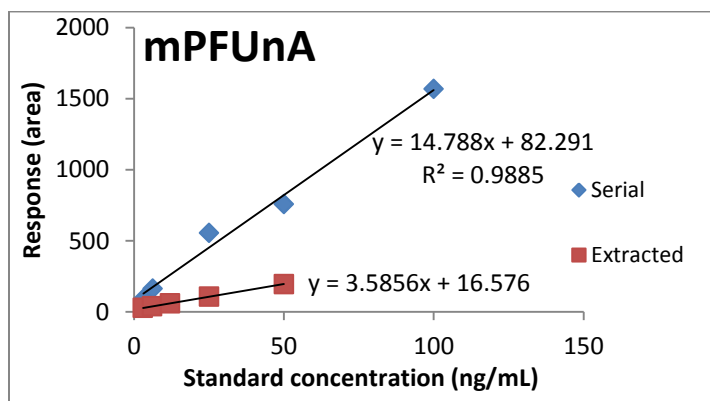
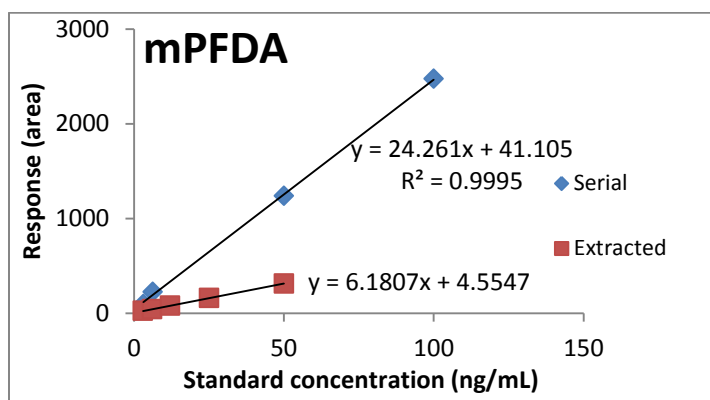
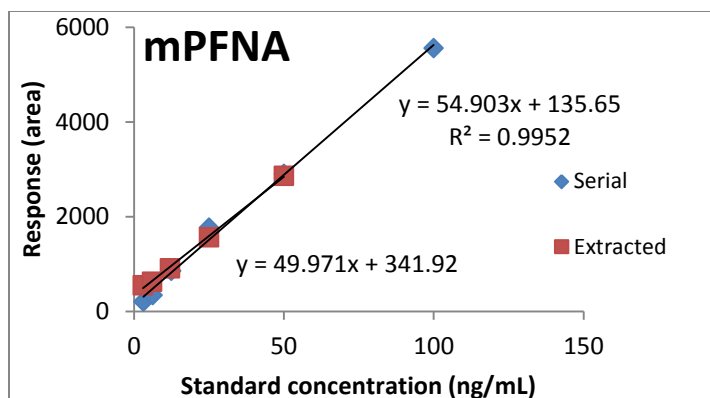
Figure A8: UPLC-MS/MS data obtained from the analysis from the SPE extractions of aqueous phase samples from the Housatonic River and LIS surface water samples obtained during the spring high river discharge survey (survey 2, May-June 2013) was initially calculated using a serial dilution curve, resulting in inconclusive results which were not consistent with either recovery standards or method performance standards. With the new mass labeled isotope mix now including mPFOA and mPFOS which had previously been utilized as instrument response standards, all calibration calculations performed were now performed using external rather than internal standards. A comparison of calibration standards prepared by SPE extraction vs. serial dilution found significant differences, leading to either under and over estimation of PFAAs in the samples, and results incongruous with other QC standards. Following evaluation of the two calibration curves, the Housatonic River survey 2 aqueous phase samples were subject to re-analysis using the data obtained from comparison of serial dilution standard curves to SPE extracted curves, under the assumption that the differences between extracted and serial curves were consistent. Results obtained for the re-analysis of these samples were vindicated by appropriate values for method performance check samples. The comparative analyses of SPE extracted vs. serial dilution standard calibration curves are shown below in the following graphs (1-24):

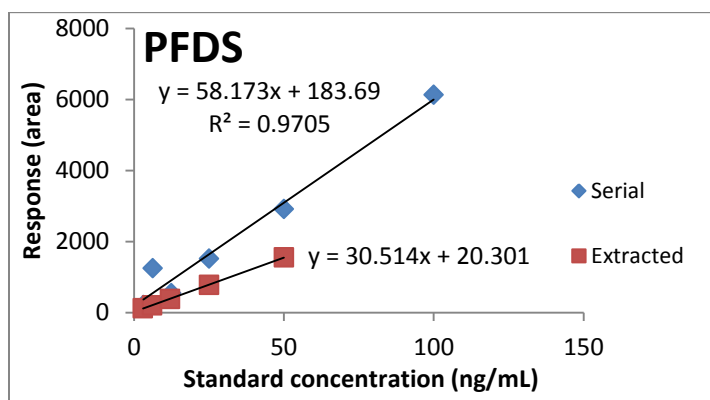
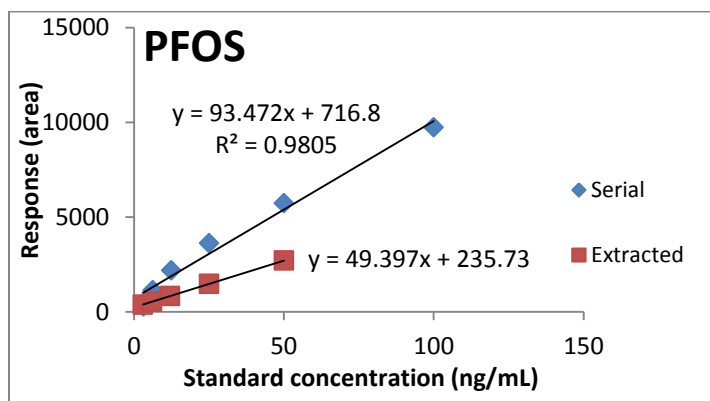
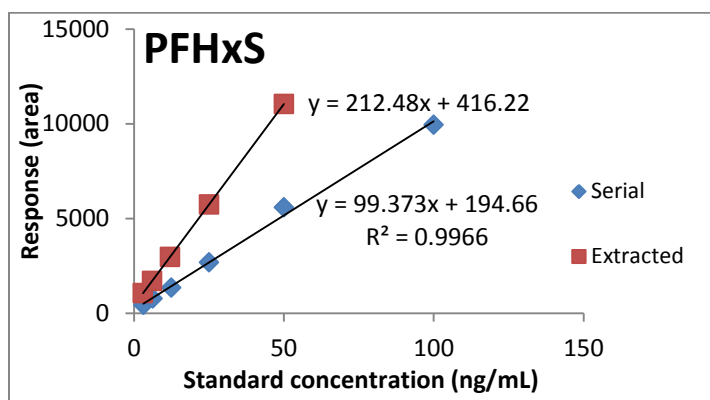
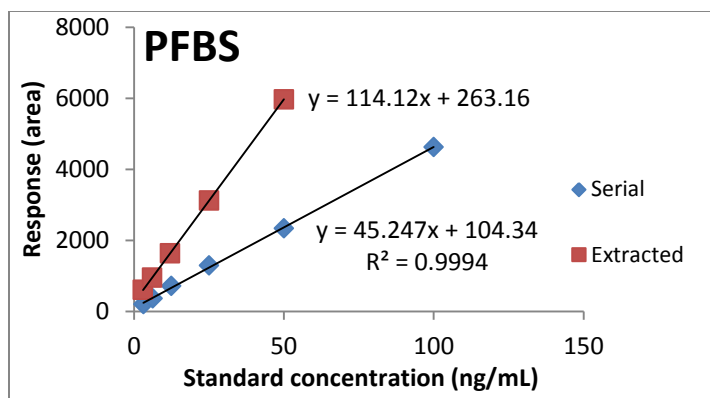












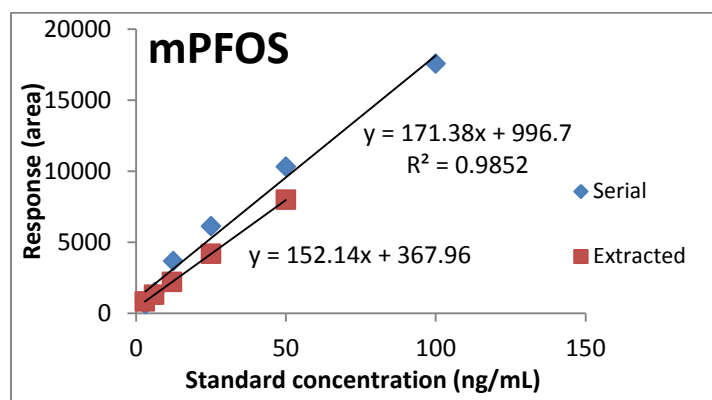
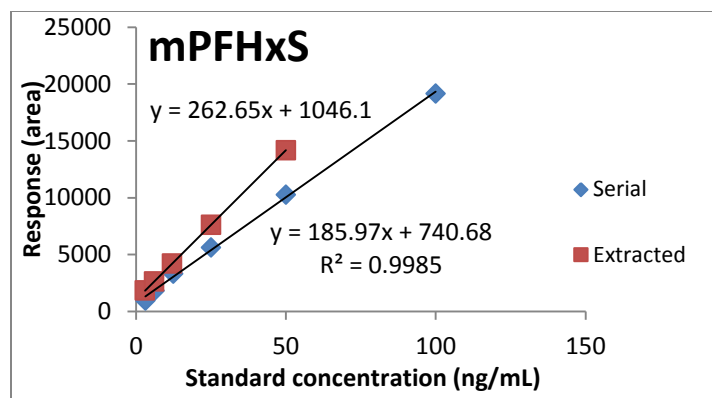


Table A12: Detailed information concerning the amount of recovery standards detected in samples obtained from the Housatonic River and LIS during the summer low river flow survey 1, July 2012. Samples with recovery standards recovered in the range of 70%-130% were not adjusted. Samples with recovery standards <70% were adjusted up to 70% recovery. Samples with recovery standards >130% were adjusted down to 100% recovery.

Sample/extraction	n	% Recovery of mass labeled standards %Standard Deviation %max-%min		
		¹³ C-PFHxA	¹³ C-PFDA	¹⁸ O-PFHxS
Aqueous phase SPE	46	98	61	64
		39	28	28
		39-194**	22-137	28-146
Solid phase- SPM- PP extraction	23*	144	78	111
		42	28	34
		61-217**	30-152	44-215**
Oysters- SPE extraction	6	129	108	49
		34	40	22
		83-177**	53-162	33-84
Sediments	16	168	99	118
		75	45	55
		62-328**	30-190	33-234**

*5 samples had elevated amounts of recovery standards ranging from 223%-511% for ¹³C-PFHxA, 119%-279% for ¹³C-PFDA, and 203%-360% ¹⁸O-PFHxS. These higher recoveries were assumed to be a result of matrix effect causing ion enhancement, therefore analogue native PFAAs detected in these samples were adjusted down to 100% recovery.

** Despite all efforts to remove matrix contaminants, some samples had high recovery standards. This was attributed to matrix effect causing ion enhancement, therefore all target PFAAs were assumed to be affected in the same manner, and all recoveries corrected down to 100% using the recovery standards.

Table A13: Details on the amount of recovery standards detected in samples (2L) obtained from the Housatonic River and LIS during the spring high river flow survey 2, June 2013 is given for recovery standards with native analogue target PFAAs detected in field samples. Data for the aqueous phase extractions was obtained using derived extracted calibration standard curve based on the assumption that the differences between the extracted standards vs serial dilution curves are consistent. Due to the data being based upon this assumption, samples were not adjusted for recovery using the recovery standards. Data was only deemed acceptable in each individual peak had a S/N ration >10.

* not detected in samples

Sample/extraction	n	% Recovery of mass labeled standards			
		%Standard Deviation			
		%max-%min			
		[¹³ C ₄]-PFBA	¹³ C-PFHxA	[¹³ C ₄]-PFOA	[¹³ C ₄]-PFOS
Aqueous phase SPE	35	26	54	48	73
		8	20	29	38
		13-42	14-88	11-101	22-146
Solid phase- SPM- PP extraction	18	128*	60*	57	79
		19	10	25	38
		110-199	47-95	24-117	27-133